National Institute on Drug Abuse

95

Research

MONOGRAPH SERIES

Problems of Drug Dependence 1989

Proceedings of the 51st Annual Scientific Meeting

The Committee on Problems of Drug Dependence, Inc.

U S DEPARTMENT OF HEALTH AND HUMAN SERVICES • Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

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Editor: Louis S. Harris, Ph.D.

NIDA Research Monograph 95

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICE
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

National Institute on Drug Abuse
Off ice of Science
5600 Fishers Lane
Rockville, MD 20857

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, DC 20402

NIDA Research Monographs are prepared by the research divisions of the National Institute on Drug Abuse and published by its Office of Science. The primary objective of the series is to provide critical reviews of research problem areas and techniques, the content of state-of-the-art conferences, and integrative research reviews. Its dual publication emphasis is rapid and targeted dissemination to the scientific and professional community.

Editorial Advisors

NIDA Research Monograph Series

Parklawn Building, 5600 Fishers Lane, Rockville, Maryland 20857

FOREWORD

This volume contains the scientific papers presented at the 51st Annual Meeting of The Committee on Problems of Drug Dependence. The meeting was held in Keystone, Colorado in June 1989. The National Institute on Drug Abuse is pleased to make these exceptional contributions to the study of drug dependence available to the public as it has for papers presented at other meetings of the CPDD. Once again, the topics cover a wide range of research interests, from the anatomy of brain cells to the prevention and treatment of drug dependency.

Of particular interest at this CPDD meeting, though not presented in this volume, was the discussion of the formation of a new research division within NIDA for medications development. There is considerable excitement about the concept of developing new medications for the treatment of addictive disorders, as well as brain and behavior dysfunction. The programs funded by NIDA through this new division will stimulate new research in the neurosciences and pharmacology by members of the CPDD and other scientists. I look forward to the volumes of proceedings of coming CPDD meetings, in which we can expect to see the research papers that will result from NIDA's new medications development program--another example of the joint concern NIDA and CPDD in unraveling the intricacies of drug dependency.

Charles R. Schuster, Ph.D.
Director
National Institute on Drug Abuse

ACKNOWLEDGMENT

The Committee on Problems of Drug Dependence, Inc. , an independent, nonprofit organization. conducts drug testing an evaluations for academic institutions, government, and industry. This monograph is based on papers or presentations from the 51st Annual Scientific Meeting of the CPDD, held in Keystone, Colorado, in June 1989. In the interest of rapid dissemination, it is published by the National Institute on Drug Abuse in its Research Monograph series as reviewed and submitted by the CPDD. Dr. Louis S. Harris, editor of the monograph, is Chairman of the Department of Pharmacology, Medical College of Virginia.

DHHS publication number (ADM)90-1663
Printed 1990

NIDA Research Monographs are indexed in the *Index Medicus.* They are selectively included in the coverage of *American Statistics Index, Biosciences Information Service, Chemical Abstracts, Current* Contents, *Psychological* Abstracts, and *Psychopharmacology Abstracts.*

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Welcome and Current Status of CPDD

William L. Dewey

Good morning ladies and gentlemen: I am Bill Dewey, Chairman of the Board of Directors of the Committee on Problems of Drug Dependence. It is my pleasure to welcome each and everyone of you to the Fifty-First Scientific Meeting of CPDD.

On behalf of the Board, I thank Marty and Toby Adler; Ellen Geller; Marie McCain and all others who worked so hard to make sure all of the administrative aspects of the meeting go smoothly. Lou Harris and his committee did a splendid job of putting together this excellent program. Of course, thanks to the participation of all of you, the science is outstanding. The local organizing committee headed by Dick Deitrich also did a great job. Next year's meeting will be in Richmond, VA and I worry about us being able to match the ambience here. We will not be able to promise you snow capped mountains outside your bed room window, but will guarantee you the very best in southern hospitality.

Now, I want to relate to you the current activities of CPDD and to let you know the status of a number of the important ongoing projects.

CPDD has one major objective and that is to rid the world of illicit drug use. We are convinced that the only way that this can be done is by decreasing the demand. This will never be accomplished without a significant increase in knowledge which only can be generated through research.

CPDD has three specific objectives. The first is to hold excellent scientific meetings in the field of substance abuse. We currently hold an annual meeting and at appropriate times other meetings on a specific topic related to drug abuse. There has been a major change in our annual meeting in recent years. We now have multiple symposia, each dealing with important aspects of drug abuse. The scope of these symposia includes molecular biology, chemistry, pharmacology and clinical studies. Each symposia is on the "cutting edge" of its discipline.

We often hold specific topic meetings such as the one co-sponsored by CPDD and the American Society for Pharmacology and Experimental Therapeutics (ASPET) entitled, "Scientific Perspectives on Cocaine Abuse". This meeting was held in January 1987. Reprints of the document generated as a result of that meeting, are available from the executive office of CPDD. Last year we held a meeting on the appropriate methodologies for the testing of medications expected to have abuse potential or dependence liability. In 1991 we will co-sponsor a meeting devoted to the pharmacology of kappa receptors.

The second specific objective of CPDD is to conduct a testing program to determine the abuse potential and addiction liability of compounds. CPDD serves as an honest broker in this regard for compounds submitted by the pharmaceutical industry, chemists in academia and in governmental laboratories. It is this function of CPDD that separates it from all other scholarly scientific societies or organizations. In recent years the testing functions of CPDD have and are continuing to evolve and expand.

The opioid program is long standing and well known to most of you. In the past year we have initiated a new program for the assessment of the abuse liability and dependence potential of stimulants and depressant compounds. This program is running very well. Four laboratories are participating and approximately 20 standards and seven new compounds have been tested to date.

A very exciting current function of CPDD is to expand the testing activity to human testing. Mechanisms and protocols are being developed for this next aspect of our attempt to provide academia, government and industry with an independent clinical testing facility for all new compounds.

The third specific objective of CPDD is to be ready to serve in a consultative fashion for governmental agencies at the local, federal and international levels. The following are a few examples of how this is done. First and foremost the data generated from our testing program is important information used by FDA and WHO when scheduling decisions are made.

Secondly, CPDD has worked hard to convince congressmen and the executive branch of government that additional and a consistent level of high funding is needed to provide the basic, clinical, prevention, treatment and all other types of research that is so badly needed in the area of substance abuse. Members of CPDD have testified before the appropriation committees of both Houses of Congress in this regard. We feel we have been somewhat successful in the past but recognize the need to continue and increase this very important effort which benefits biomedical science in government and industry as well as academic laboratories. CPDD has responded to the new APHIS guidelines on the humane use of laboratory animals in research

and have taken a lead role in this important area with politicians at all levels.

We are in the process of responding to a specific request from the Select Committee on Narcotic Abuse of the House of Representatives on the issue of possible legalization of illicit drugs. They have asked us for scientific input which is needed for the lawmakers to base their decision on this very important question. These are a few examples of what the organization is doing. In closing this part of my comments, I will tell you that CPDD is constantly improving its scientific meeting, the testing programs, and its interaction with various governmental agencies. I hope I have convinced you that CPDD is a vital organization, functioning well in many aspects.

At its meeting this week the Board of Directors voted for a major change in the structure of CPDD. With a target date of July 1, 1990, CPDD will become a membership organization. Because of the unique functions of CPDD as described earlier, it is not envisioned that CPDD will be like many other organizations with open membership but might have a format somewhat similar to that of the American College of Neuropsychopharmacology (ACNP). We expect to differ from ACNP by having our meetings open to the entire scientific community. We feel this is essential to maintain progress in the multiple research efforts to solve this important problem. The objective is to maintain the unique and important functions of CPDD but to provide a home organization, if you will, for the many excellent scientists making important contributions to the multiple aspects of this important field. The details and new by-laws will be worked out during this year.

At this time I am very pleased to recognize six members of the Board of Directors of CPDD who have each contributed significantly to the function of their organization as board members over recent years. Their terms on the beard have now ended. As is our custom, each will be presented with a certificate as an indication of our gratitude for their significant contributions to CPDD. The six members who will retire after this meeting are: Drs. Richard Deitrich; Sheppard Kellam; Herb Kleber; Mary Jeanne Kreek; Horace Loh and Everette May.

All of us are indebted to them for their excellent scientific contributions to this important field and also for their hard work and multiple contributions to the objectives of the Committee on Problems of Drug Dependence.

Richard Deitrich: Dr. Deitrich brought his special expertise to the Committee in the area of alcohol research. Dr. Deitrich served as the Chairman of the Nominating Committee for the Committee on Problems of Drug Dependence, and also was in charge of local arrangements for this meeting at Keystone, Colorado.

His contributions to the Committee in both of these regards were exemplary, and we are very appreciative of his efforts.

Shep Kellam: The expertise of Dr. Kellam in the area of public health was of immense importance to the Committee. Clearly, drug abuse is a problem which affects many people in our country, and the perspective from the public health point of view was essential in the deliberations of the Committee. Dr. Kellam's interest and expertise in interacting with various advocacy groups, such as AARP and NAMI, were particularly important to the functioning of the Committee on Problems of Drug Dependence during his tenure on the Board of Directors.

Herbert Kleber: Dr. Kleber brought a great deal of clinical expertise to the Committee. His insightful knowledge of treatment of patients who abuse various types of drugs was especially important to discussions of significant issues before the Board. He was a major contributor to our Clinical Testing Committee, which is a relatively new endeavor of the Committee, and the expertise of Dr. Kleber and his colleagues made this important function possible.

Mary Jeanne Kreek: Dr. Kreek served the Committee in many ways. Her great knowledge of the effects of drug abuse, both in basic and clinical science, were particularly valuable to the discussions of the Board of Directors, as well as in many symposia and scientific meetings. Dr. Kreek also served the Committee as Chairman of the Board of Directors for two years, and did an exceptionally good job in bringing forward the many efforts of the Committee during that time. She also filled in as interim Executive Officer of the Committee, simultaneously while serving as Chairperson. Her vast energy and exceptionally good judgement were tremendous assets to the continued progress of the Committee on Problems of Drug Dependence. All workers in this field, and especially all those who are struck with the complexity of the abuse of drugs, should be appreciative of the multiple efforts and the marked expertise of Dr. Kreek. The organization prospered well under her outstanding leadership.

Horace Loh: Dr. loh has contributed to all the functions of CPDD but he has been especially active on its program committee and by organizing symposia for the scientific meetings. His expertise as a biochemical and molecular pharmacologist has been of great value to the Board of Directors of the Committee on Problems of Drug Dependence. His work and the work of his colleagues has greatly increased the quality of the scientific meetings of the Committee.

Everette May: Dr. May, who actually retired from the Board of Directors last year, continued his outstanding, long-lasting commitment to CPDD by co-authoring the history of the organization. Reprints of this document are available from the executive office. The many contributions of Dr. May to this field, and particularly to CPDD, are obvious to us all and are

so extensive that they can not be described here.

We thank each of these matters of the Board for their outstanding contributions to the Committee on Problems of Drug Dependence.

MOTOR:

William L. Dewey, Ph.D.
Associate Provost of Research and
Graduate Affairs
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298

Animals in Research on Addictive and Mental Disorders: Foundation of the Quest -for Knowledge

Frederick K. Goodwin

Let me begin with two observations: 1) Biomedical and behavioral research is critical to the success of this Nation's war against drugs, and 2) Basic research on drug abuse (along with alcoholism and mental illness research) has been targeted by the radical animal rights/stop research movement, presumably because they feel they can play on public attitudes that these problems are not fundamentally biomedical. In light of the implications of these observations to the concerns of the CPDD, I plan to focus my remarks on the animal rights issue.

I will make some general comments about the aims of the animal rights movement, describe strategies the movement is using to accomplish its ends, and suggest what I see as needed responses from the research and treatment communities.

To date, various institutions including government, professional groups. patient groups, and academia have essentially misread the agenda of the animal rights movement. We in the research community have tended to respond to the challenges of the animal rights activists as we have been trained, which is intellectually. In fact, we are dealing with an anti-intellectual movement whose fundamental philosophical premise is incompatible with the humanistic values of the health professional. Tactically, the movement pursues its radical agenda with political sophistication, passion, and a very effective appeal to nearly universal sentiments about animals. We must respond on all those levels in addition to the more purely intellectual level.

The Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) is actively responding to the threat to biomedical and behavioral research posed by the animal rights movement. Mental and addictive disorders involve complex human behaviors that overlap with and can be mistaken for normal, voluntary misbehaviors. Consequently, we are vulnerable to the tacit implication that our research substitutes biologically or psychosocially reductionistic explanations for behavior which should be simply a matter of individual responsibility. It certainly is much easier for groups opposed to research to mislead the public about complex behavioral disorders than, for example, the need to develop a new heart valve or to cure a cancer.

It is essential that all of us educate ourselves about the animal rights movement and understand the urgency of public education, whether or not we are directly engaged in animal research. Since the animal tights movement is fundamentally anti-intellectual and anti-science, each of-us is threatened by its success.

Animal Welfare vs. Animal Rights

It is important to distinguish clearly the animal welfare and animal rights movements. Animal welfare is a traditional, well-respected part of mainstream advocacy in this country and in most of the western world. Based on the philosophical premise of responsible stewardship of animals, animal welfare encompasses humane care. Traditional animal welfare advocates operate on the premise that humans are responsible for animals, not that animals have intrinsic rights. This latter notion conflicts not only with our cultural and religious values concerning the sanctity of human life and our special dedication to human rights, but also with the foundation of our entire legal system.

Traditionally, the animal welfare groups such as the Society for Prevention of Cruelty to Animals (SPCA) have pursued their goals of preventing cruelty and unnecessary suffering through such efforts as helping abandoned strays and pets, building and maintaining shelters, sponsoring adoption and neutering programs, and conducting pet care education. The SPCA and similar groups typically have avoided the question of use of animals in research, but, when pressed, have not opposed it.

The contemporary animal rights movement, by contrast, holds that all beings which have a capacity for suffering have equal "interests." This position, developed in the mid-1970s by an Australian philosopher, Peter Singer, was presented in the book Animal Liberation: A New Ethic for our Treatment of Animals. As a "bible" of the animal rights movement, Singer's work is the basis for the movement's assertion that the claim by humans of special rights is "speciesism," a moral equivalent of racism and sexism.

Currently, some 10 million Americans, allied loosely through more than 400 individual groups, are counted in the animal rights movement. Many of these persons are genuinely concerned about human responsibilities toward the welfare of animals. Yet they have been misled by the leaders of the animal rights movement who have purposely obscured the-underlying philosophy and ultimate aim of the movement--the elimination of all use of animals in research.

In choosing not to confront directly society's widely-held sensibilities about the primacy of human life and health, but rather to rely upon specific objections to the use of animals in particular research areas, the movement has misled those who would be opposed to its fundamental philosophical premises. By putting the research community on the defensive (a position which we unfortunately reinforced) the movement was able to present itself as reasonable and constructive.

This image has enabled animal rights activities to infiltrate and radicalize many traditional animal welfare organizations. Through "hostile takeovers" of animal welfare organizations and sophisticated fund-raising, activists have come into control of immense resources which now are being channelled into the stop research movement. Sadly and ironically, animal welfare activities are being curtailed by a lack of funds. Shelters must kill some 16 million animals each

year, for example. in part because adoption and neutering programs have been neglected while resources are diverted into the stop research movement.

Early on, the image of reasonableness encouraged the research and academic communities, as well as government agencies, to respond reflexively--and, often,. defensively--to secondary anti-research arguments, while ignoring the fundamental philosophical or moral issues. When animal rights activists claimed that cruelty to animals in biomedical research was commonplace, the field acquiesced to demands for greater regulation of its operations, thereby seeming tacitly to agree that the isolated and distorted examples publicized by animal rights activists were typical of research lab procedures.. Reports issued by the Office of Technology Assessment seemed to imply that while animal research remained necessary now, further, progress would eventually supplant that necessity, an implication that was further reinforced when the National Institutes of-Health (NIH) agreed to substantially increase its funding for special centers to develop more alternatives to the use of animals in research.

It soon became evident that attempting to appease the animal rights activists was a profound mistake; as we accommodated and compromised--and projected a sense of apology for our research procedures--the animal rights movement became more strident and more successful.

The Cultural Climate and Animal Rights

As was true of other eras in which strong animal rights sentiments emerged--during the period preceding World War I, for example, or in 1930s Germany--specific features of the cultural climate in the U.S. and other developed countries in recent decades fostered the rapid growth of the animal rights movement. The late 1960s and ’70s were characterized by a subtle but pervasive sense of demoralization or nihilism about the human condition. This attitude set the stage for a confusion between human and other forms of life, which is at the core of animal rights.

In the U.S., the political and social turmoil triggered by three assassinations and by Vietnam undermined willingness to invest in the Nation’s infrastructure, perhaps reflecting an erosion of confidence in the future and in our ability to improve it. The legacy of that era is found today in the deteriorated condition of infrastructure components ranging from transportation to education to biomedical research. With, respect to research, a marker is the diminishing percentage of GNP devoted to research and development; another is the proportion of health care costs invested in research, which has declined nearly 50 percent over the past 25 years.

Another index of demoralization in the U.S. was the widespread mistrust of establishments and institutions--including university research centers--in the post-Vietnam, post-Watergate era.

A perhaps related measure of demoralization was seen in efforts during that period to eliminate any suggestion of “moral tone” from educational processes. As noted by Allan Bloom and others, traditional absolutes of education frequently were abandoned in favor of “cultural relativism.” Unfortunately, science doesn’t do well in a relativistic atmosphere--one has to master it in order to debate it.

It is not difficult to see linkages between the trend toward relativism, the contemporary dismal quality of science education in U.S. primary and secondary schools, and the growth of the animal rights movement. Today, only four percent of the teachers in our primary schools have had a science course beyond high school; only seven percent of our high school graduates now meet even minimum criteria for scientific literacy.

Not surprisingly, a substantial proportion of the membership of the animal rights movement consists of young people who tend to substitute sentiment for reason. When pressed to discuss their position, they say, in effect, "Because I feel strongly about not using animals in biomedical research, it's true for me." Many students simply don't understand the anti-intellectual, anti-authority, and anti-institutional attitudes which underlie the "arguments" of the animal rights movement.

Equally worrisome is that biomedical science means little to a generation for whom the conquest of infectious diseases with vaccines is history. In school, kids are taught that health is solely a matter of prevention: no smoking or drug use, safe sex, good nutrition. The idea that killer diseases still exist is hardly urgent to young people--disease and death are remote, something for their grandparents to worry about.

The Secondary Anti-Research Arguments

In addition to being aware of and understanding the philosophical impetus behind the animal rights movement, those of us who must defend research must also be able to respond to the movement's secondary anti-research arguments; these essentially fall into one of the six categories discussed below.

I. "Animal research is inherently cruel." Animal activists repeatedly use and often distort a few isolated cases. In fact, we might be encouraged that activists are so dependent on the publicity value of two dated instances, one involving the Silver Spring monkeys and, another, the University of Pennsylvania Head Injury Lab. In the Appeals Court, the principal investigator on the Silver Spring project was acquitted on all charges of animal cruelty; indeed, during the court proceedings, witnesses were prepared to testify that an activist member of People for the Ethical Treatment of Animals (PETA) who had worked at the facility had subjected an animal to severe distress in order to get a photograph that is now being used in fund-raising by the animal rights movement. In the other instance, activists broke into the University of Pennsylvania's Head Injury Research Lab in 1985, stole videotapes made over the course of many months, and selectively edited these into a short, exploitative tape which depicted instances of insensitivity and carelessness. Sanctions imposed by NIH on the University for violations of the Guide for Care and Use of Laboratory Animals have been dishonestly used by the movement to convey the message that the research was not only inherently cruel but medically useless.

Use of anesthesia, standard in almost all invasive research, is especially important in behavioral research, where undue pain and distress can severely distort the validity of findings. In the few studies where pain per se is being studied and anesthesia is not used, "thresholds" for pain are the most informative research measure; sustained administration of genuinely painful stimuli would be counterproductive to the overwhelming majority of research goals.

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Research is one of the most highly regulated activities in our society, and animal research is subject to stringent regulatory requirements. The current proposal for revised federal regulations governing animal research runs to 132 pages of highly detailed instructions which are subject to continuous revision by a committee that includes non-scientists. Today, anecdotal reports from investigators suggest that it actually is easier to receive approval to conduct certain research with human subjects!

II. "Animal research is wasteful and duplicative." As members of the scientific community know all too well, competition for federal research funding is intense. Grant applications are extensively reviewed, first, for scientific merit and relevance by scientists with expertise in the area of proposed research and, subsequently, by a statutory Advisory Council to ensure that the proposal is responsive to public health demands and the mission of the funding agency. Given limited research resources, scientists who peer review each others' applications obviously are not going to approve frank duplication.

Grantees must certify, moreover, that they are in compliance with all federal animal welfare policies; this requires documentation from the Institutional Animal Care and Use Committee, which must include as members a veterinarian, a researcher, a non-scientist, and a member of the community not affiliated with the university site of the proposed research.

Recent legislative efforts, such as the Torricelli Bill, which has been promoted as a "pro-science" measure to help prevent duplication, would overlap the existing, proven system of dual review with a process that is essentially adjudicatory in nature. The authors of "protections" such as this fail to see that replication, a form of duplication, is essential in the task of converting experimental findings into "truth," and they fail to understand how new scientific knowledge comes from multiple, overlapping findings, each building on the other. If enacted, the Torricelli Bill would be a disaster for biomedical science.

III. "Animal research diverts funds from treatment." This argument, unfortunately seductive to a few naive treatment professionals, represents an effort by the animal rights movement to balkanize the research and treatment communities, by pitting those who generate new knowledge against those who apply the information in clinical settings.

The data, however, do not come close to supporting the claim. For every $100 the Federal Government spends on health care generally, less than 40 cents supports animal research. For mental and addictive disorders exclusively, the figure is 2 cents per $100 in treatment costs!

Uncontested, this argument is particularly threatening to the research fields supported by ADAMHA. The Federal Government, primarily through ADAMHA, supports approximately 85 percent of all research on mental and addictive disorders conducted in this country. While adding all funds spent by ADAMHA on animal research to current Federal outlays for mental health and substance abuse care would not even create a ripple in the services budget, the impact on research opportunities would be immense, virtually eliminating the possibility of a scientific breakthrough based on animal research--a breakthrough such as the discovery of lithium for treatment of manic depressive illness.

IV. "Animal research is unnecessary to medical progress." Given the widespread scientific illiteracy of the target audience, this is a persuasively deceptive argument. Because there typically is a lag of at least 10 years between the conduct of basic animal research and its clinical application, the relevance of basic studies to health care often is difficult to perceive. This is particularly true of basic studies in the fields of mental and addictive disorders.

In fact, virtually every advance in medicine either originated in basic animal research or involved animals in a key part of its development. In the mid-1970s (prior to the current controversy), Comroe and Dripps (1) polled clinicians in cardiovascular-pulmonary medicine as to what they viewed as the medical advances which were most helpful to their patients. Responses included open heart surgery, drug treatment of hypertension, cardiac resuscitation and defibrillation techniques, oral diuretics, chemotherapy and antibiotics, early diagnostic methods, prevention of poliomyelitis, and others. All of the advances were shown by the authors to have depended on animal research at critical stages of their development.

Four of the top ten ranked advances, moreover, were shown to have originated in unrelated fields, a finding that effectively rebuts the frequent demand of animal rights activists that every research project must be justifiable in terms of a predictable health application. By this yardstick, most contemporary medical advances never would have happened.

Animal research is no less critical to advances in the treatment of mental and addictive disorders, a fact that assumes added significance given the manner in which the animal rights movement has targeted research on "behavioral" disorders. Lithium, used today to treat successfully some 75 percent of patients with manic depressive illness, was a direct and unexpected product of animal research. Before lithium was introduced, a patient with this illness would, on the average, spend one-fourth of his adult life in the hospital; more than one in four with the illness died by suicide. Today, 80 percent of manic depressives never see the hospital and the suicide rate among persons being maintained on lithium is close to that of the general population. In the U.S. alone, lithium has yielded direct treatment cost savings of $12 billion over the nearly two decades since receiving FDA approval.

A timely example of a direct contribution of animal research to drug abuse treatment is seen in the recent clinical evaluation of carbamazepine, an anticonvulsant drug, in treating cocaine addicts. This innovative approach stems from research conducted at the NIMH that was concerned with the tendency of some patients with manic depressive illness to cycle rapidly between episodes of mania and depression. At the turn of the century, Emil Kraepelin had observed that the illness could initially be triggered by stresses; later, Bob Post and I had hypothesized several years ago this reflected the activation of a genetic predisposition and that, once activated, the illness assumes its pattern of spontaneous recurrences. Post drew an analogy between this and the process of electrical kindling in the CNS; that is, the tendency of a highly regulated system, such as the limbic system, to respond more and more measurably to a repetitive electrical charge and, eventually, to produce that response spontaneously, in the form of a seizure, in the absence of the stimulus.

Dr. Post subsequently determined that a kindled seizure could be induced pharmacologically, through repeated but intermittent administration of cocaine

and that the seizure--whether induced electrically or pharmacologically--could be arrested by the anticonvulsant carbamazepine. The medication has since been shown to be an effective treatment for a substantial number of lithium-resistant rapidly cycling manic depressive patients.

Upon hearing reports of Post's work, Jim Halikas, a psychiatrist at the University of Minnesota, immediately initiated an open clinical trial of carbamazepine in a small group of hard-core cocaine addicts (2). The medication yielded dramatic results: cessation of use in about half of the subjects and significant reductions in frequency of use among others.

These preliminary findings, now being evaluated in controlled studies, are particularly noteworthy for several reasons. First, is the demonstration of unexpected benefits that result from basic animal research; in the absence of the theoretical basis provided by Post's animal work, it might not have occurred to those in the cocaine treatment community to work with an anticonvulsant. Second, the link between the animal observation and the clinical trial was both direct and immediate--there was no 10 or 20 year lag between the observation in animal studies and the preliminary application to a clinical problem. And third, the finding underscores the relevance of research on basic brain processes in animals to human brain disorders such as addiction.

V. "Modem alternatives can replace animals in research." This version of the "we don't need animals" argument holds that alternatives to the use of animals in research exist. Regardless of how little people may know about science, most are familiar with such buzzwords of the argument as "computer modeling" and "tissue culture," and most have passing familiarity with such technological innovations such as scanning devices. With respect to computer modeling--an "alternative" particularly appealing to young people who are caught up in the romance of computers--the fact is deliberately obscured that computer modeling living systems first requires actual data from living systems; that a computer model is nothing more than a hypothesis, a way of formalizing scientific beliefs in a way in which they can be quantitatively tested. The fact that tissue culture cannot substitute for the complexity of an animal, particularly an organ like the brain, is similarly obscured. And those who claim that devices such as PET scanners offer a substitute to the use of animals conveniently overlook the many years of animal research that were required to develop the tracer technology that made the PET scanner useful.

VI. "Lost pets held in pounds are at risk for research use." More than 90 percent of all animals used in research are rodents, rats and mice principally. For every cat and dog used in brain/behavioral research, 7,000 are killed by pounds or shelters, a number that is increasing for several reasons. One, noted above, is the reduction in funds available for adoption and neutering programs traditionally conducted by animal welfare groups as resources are channeled to the animal rights agenda. Also, when State and local laws prohibit the sale of pound animals to research facilities, these animals will be killed. At the same time, animals must be specially bred for research purposes, at considerably more cost.

Impact of Animal Rights on Behavioral and Biomedical Science

Even within the research community, the view often is expressed that the animal rights movement initially was helpful in, that it increased sensitivity to animal welfare. But the helpful phase is now long gone, replaced by the fear reflected

in the "bunker mentality" of individual scientists and universities. We already are seeing a sharp decrease--62 percent decrease over the past two years--in the number of drug abuse studies using primates. Given the urgency of the drug abuse crisis and the scientific consensus on the need for animal models, it is difficult to ascribe such a large decrease to anything else but fear.

The animal rights movement also has had a substantial impact on the costs of conducting research. Implementation of new animal care guidelines has been estimated at $1 billion in the first year; this does not account for costs that will be incurred in meeting recent requirements addressing the "psychological well-being" of animals in research facilities. A billion dollars is roughly equivalent to a 17 percent cut in the research budgets of the NIH and ADAMHA, the ultimate source of most of the funds.

An ominous long-term impact of the movement is the dramatic decline in "hands on" experience with animal research in education, including the medical schools. A recent survey found that 42 percent of medical graduates planning to pursue careers in clinical practice questioned the value of animal research, a reflection of attitudes that are indirectly being nurtured in schools at all levels. Motivated in part by attempts to appease activists, some mainline educational organizations such as the National Association of Biology Teachers have passed resolutions outlawing the use of animals in class, and even in some medical schools, students are allowed to opt out of participation in laboratories where they gain "hands on" familiarity with animals in the lab.

Without a massive effort to reinstate basic principles of science education, the life sciences not only will be increasingly handicapped in the recruitment of students in the future, but also will lose the current public support as today's students grow up. A recent National Family Opinion survey commissioned by the Foundation for Biomedical Research found that 77 percent of the American public support the use of animals in biomedical research; that 70 percent support basic biomedical research, even when it will not lead directly to the cure or treatment of a specific disease; and that 65 percent oppose organizations attempting to stop the use of animals in research or product safety testing. Present trends in the educational system suggest, however, that this level of support will erode in the future.

What Must Be Done

The fundamental philosophy of the animals rights movement is becoming increasingly well-understood, the strategies employed by the movement are clear, and the impact, to date, is considerable. If we are to ensure the continued vigor and productivity of the biomedical and behavioral research enterprise, the government, universities, physicians, and patient organizations must work proactively and collaboratively.

The pharmaceutical industry and other high-tech enterprises that depend on basic research must also become more actively involved. The animal rights movement currently spends more than $52 million dollars annually to stop animal research. The biomedical research establishment in its entirety spends about $1 million per year to conduct public education and counter the misinformation disseminated by the movement. Although most of these funds are provided by pharmaceutical companies, the amount is minuscule in comparison to the many millions of dollars a company typically will spend to market one product derived from animal research. Most companies are

unwilling to assume high individual visibility in defense of animal research, given the perceived risks of boycotts and demonstrations, confrontations with stockholders, and death threats to senior corporate officers. These concerns would be minimized if the industry acted in unison with a single action plan that all relevant businesses would support.

Similarly, universities must be encouraged to stand fast in the face of harassment from the animal rights movement. A strategy being employed by movement activists is to target a specific research project at a given university for protest demonstrations. In a well-publicized case, activists intimidated university officials into declining a federal research grant to a senior investigator after the application had been reviewed and approved, both for scientific merit and animal welfare.

In the wake of this incident, ADAMHA determined the need to assess carefully any research grant applications from a university showing any tendency to yield to animal rights pressures. University administrators must be encouraged to appreciate the responsibilities which the institution assumes in competing successfully for federal research funding. The return of a grant for non-scientific reasons--for example, to keep the campus quiet or to placate a small vocal minority of alumni--wastes the resources devoted to the review process and conveys the message that the process can be undermined for reasons that have nothing to do with research. Universities that bow to "stop research" pressures exerted by animal rights activists must understand that they are jeopardizing their eligibility for all ADAMHA grants, not just those using animals. Unless federal funding agencies set up a counterpressure, it is possible that an increasing number of universities will give in to the demands of the movement one at a time. At this writing, the National Institutes of Health has not formulated policy in this particular area of concern; ADAMHA has taken the initiative because research on mental and addictive disorders has been specially targeted by the animal rights movement.

Within the research community, mechanisms are needed that will provide incentives for scientists to devote time to nurturing and protecting the enterprise. Currently, extensive service as a spokesperson for the use of animals in research takes time away from the bench or clinic and may be perceived as a handicap in competition for grants. We must explore novel means of rewarding those academicians who are inclined and willing to do advocacy for animal research and other areas of infrastructure concern.

And the federal life sciences agencies, principally NIH and ADAMHA, must do a great deal more in direct public education efforts. NASA provides a good model. The agency reaches out to primary and secondary level students to encourage their interest in curricula necessary for careers in the space program. There is virtually no comparable effort on the part of the federal agencies responsible for the life sciences, and it is evident that the research community can no longer take the interest of future generations of students for granted.

Scientists who are interested in science advocacy and public education must develop the necessary skills. Given the tenor of the animal rights controversy, media training--particularly in the handling of confrontational interviews-is useful.

If the research community does not address the threat of the animal rights movement with a single voice, the movement will continue to grow in size and

effectiveness. If we allow this to happen, sooner or later, animal research and all that it can provide for the health of our people with be a thing of the past.

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AUTHOR

Frederick K. Goodwin, M.D.
Alcohol, Drug Abuse, and Mental Health Administration
Rockville, MD

Implication for Research of the 1988 Anti-Drug Abuse Act

Charles R. Schuster

OPENING REMARKS

Thank you. Dr. Dewey. It's a pleasure to be with you again at CPDD's Annual Meeting. As always, I look forward to this opportunity for drug abuse researchers to share the progress that is being made toward understanding and controlling the drug problem, and to discuss the directions that drug abuse research should be taking in the future.

Several of these directions of research are now being established or impacted by a very significant piece of legislation-the Anti-Drug Abuse Act of 1988, which passed in November of last year. The Act has a total authorization level of $2.7 billion, for covering such areas as:

*Federal, State, and local drug law enforcement;

*school-based drug prevention efforts; and

*drug abuse treatment, with special emphasis on intravenous (IV) drug abusers at high risk for AIDS.

OVERVIEW OF THE ANTI-DRUG ABUSE ACT

Of the ten Titles of the Act, four have provisions that will influence efforts to reduce the demand for illicit drugs. These are:

*Title I - Coordination of National Drug Policy,

*Title II - Treatment and Prevention Programs,

*Title III - Drug Abuse Education and Prevention, and

*Title V - User Accountability.

I would like to cover Titles I, III, and V briefly, and then take a closer look at Title II, which directly impacts research programs supported by the National Institute on Drug Abuse (NIDA) and the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA).

* Title I of the Act creates the Office of National Drug Control Policy, headed by William J. Bennett, which has overall Federal responsibility for establishing the policies, objectives, and priorities for the National Drug Control Program. Director Bennett will have two Deputies-one each for supply and demand reduction-as well as an Associate Director to serve as a liaison with States and localities. The Office is responsible for reviewing and certifying each drug control budget request, and for establishing and implementing a National Drug Control Strategy. This Strategy must be completed by September 5, 1989. NIDA staff meets weekly for providing, where possible, scientific data to contribute to policy decisions and new strategies for demand reduction.

* Title III of the Act expands drug abuse education and prevention activities from the preschool level through high school, touching children in early programs such as Head Start, as well as runaways and homeless youth. This Title also funds teacher-training programs for alcohol and drug abuse prevention at the State, local, and higher education levels. Block grants will be awarded for State community-youth-activity programs.

* Title V of the Act acknowledges that uses of illicit drugs are accountable for their behavior. This is one of the more controversial parts of the new law. The Title establishes new penalties for drug possession or use; sets up the National Commission on Drug-Free Schools; develops tactics for preventing drug abuse in public housing; and extends the Drug-Free Workplace Act to contractors and grantees of the Federal Government.

THE ACT'S IMPACT ON TREATMENT AND PREVENTION PROGRAMS

Title II of the Anti-Drug Abuse Act authorizes new treatment and prevention programs and reauthorizes many existing programs-including NIDA's research activities and the Alcohol, Drug Abuse, and Mental Health Service, or ADMS, Block Grant.

NIDA's research resources have been expanded significantly as a result of the Act. Our research program has been reauthorized for three years, and it received a $30 million supplemental appropriation. With this supplement, NIDA's total budget increases from $198 million in fiscal year 1988 to $320 million in fiscal year 1990. Our AIDS budget jumps from just over $76 million in 1988 to nearly $140 million in 1990. And our budget for non-AIDS related research increases from more than $122 to

more than $180 million. Including the supplemental appropriation and Block Grant set-asides, NIDA's current non-AIDS budget increased 50 percent over the 1988 appropriation.

The number of grants we are funding is also changing. NIDA-funded AIDS research will increase from 65 grants in fiscal year 1988 to 104 in 1990. Our total non-AIDS grants will increase from 459 in fiscal year 1988 to 488 in 1990, but will drop from a high of 524 in 1989, including a nearly 17 percent decrease in new grants from fiscal year 1989 to 1990.

Of the $30 million added to NIDA's research program, $10 million were added by Representative Silvio Conte (Rep., First District of Massachusetts) to specifically fund research on the development of new medications for the treatment of addictive disorders. This money will expand our Medications Development Program for drug abuse treatment-and particularly our efforts to make LAAM and buprenorphine available as treatments for i.v. heroin addiction. Bringing such medications into use is absolutely necessary for reducing the spread of HIV infection through the sharing of needles and syringes. In addition, the Program is developing other Pharmaceuticals for treating opiate, cocaine, and PCP abuse.

The remaining $20 million of our supplemental appropriation has also made it possible to expand our research into the factors that make individuals vulnerable to drug abuse, the consequences of parental drug use on the newborn, and the development of new prevention strategies and ways to enhance existing treatment strategies.

Under the new law, NIDA is also responsible for conducting drug abuse research demonstration projects of national significance. For these, we have $7 million. These research demonstration projects will examine:

* the feasibility and long-term efficacy of programs providing drug abuse treatment and vocational training in exchange for public service, and

* the effectiveness of providing maternal care in drug abuse treatment to pregnant and postpartum women and their infants.

In addition, NIDA is providing funds to the National Institute on Justice for a research demonstration project to conduct outreach activities to i.v. drug abusers. Under this project, outreach workers will provide education on ways to prevent AIDS, and will encourage i.v. drug abusers in jails and prisons to seek treatment for such drug abuse.

Also of interest to CPDD members, NIDA and the National Institute on Alcohol Abuse and Alcoholism (NIAAA) will be providing support for clinical training programs:

* to train health professionals in the diagnosis and treatment of alcohol and drug abuse, and

* to develop appropriate curricula and materials for such training.

Although not specifically funded by the Act, NIDA has added some exciting new research training programs. NIDA 's research training has always included individual fellowships and institutional training programs. Recently, a number of other types of programs that will enhance research skills in minority populations, encourage physicians to conduct drug abuse research, and expand AIDS research training have been added to the research training portfolio.

One of the newly announced awards is the Minority Research Program Supplemental Award. This award provides supplemental support for salaries, equipment, and other items to an ongoing NIDA research grant, program project, or center grant to allow minority applicants to improve their research capabilities and to bring them into the mainstream of the NIDA research program. Current funding for NIDA's research training programs total $2 million--$1.1 million for new grants and $.9 million to continue existing grants.

A major emphasis in research training beginning in fiscal year 1990 will be in areas related to AIDS and drug abuse interactions. Research areas include epidemiology and psychosocial factors in HIV infection in drug abusers, treatment and prevention research, and preclinical studies on the. effects of chronic drug abuse on the immune system. An announcement on this research training program will soon be available. A special effort will be made during this meeting to acquaint the drug abuse community with both the new and the older programs.

To this end, the NIDA Research Training Task Force has developed a plan to introduce the research training program to CPDD members, and to introduce some NIDA trainees and fellows to a CPDD meeting. With the generous cooperation of CPDD in hosting research reports from our training programs, 28 trainees or fellows currently supported by NIDA (23 percent of the total) have received travel awards to come to this meeting and make formal presentations of their research. At least one trainee from each institutional training program and nine holders of individual fellowships are on the program this week. It seems to us to be very important for new investigators to be exposed to the breadth and depth of research areas represented at this meeting, just as it seems very important to expose CPDD members to the kinds and strengths of research training supported by NIDA.

THE ADMS BLOCK GRANT

At the administration level, the Anti-Drug Abuse Act of 1988 has consolidated all ADAMHA treatment services funds into one block grant, with $806 million appropriated for this in FY 1989. Congress has mandated specific "set-asides" for these funds. Within the total block grant appropriation, $125 million are supplemental substance abuse funds for FY 1989. The Act requires that each State must spend not less than 50 percent of these funds on treatment for i.v. drug abusers, unless they receive a waiver from the Secretary of the Department of Health and Human Services. In addition, a certain portion of the States' remaining funds in fiscal years 1990 and 1991 for drug abuse must be allocated to treatment for i.v. drug abusers. The States may use the supplemental funds to:

* develop, implement, and operate treatment programs for i.v. drug abusers;
* train drug abuse counselors and other health care providers to provide treatment; and
* conduct outreach activities to encourage people to seek treatment.

Congress also mandated that the States use ten percent of their block grant allotments for programs and services designed for women-especially pregnant women and women with dependent children-as well as demonstration projects to provide residential treatment services to pregnant women.

Prior to the Act, the States were prohibited from allowing programs to use Federal funds for construction purposes. The new law now provides a waiver mechanism so that substance abuse funds can be used for construction. ADAMHA will be issuing guidelines for this provision, as well as for a requirement that States establish a revolving fund to make loans from appropriated funds for housing for recovering substance abusers.

One important provision of NIDA in this Act is a five percent set-aside in fiscal year 1989 for data collection, technical assistance, and health services research for alcohol, drug abuse, and mental health areas. From this, NIDA and NIAAA will receive a total of $19.5 million to be used for data collection activities and services research. NIDA will devote $1.75 million to services research and, in conjunction with NIAAA, $16 million for data collection.

The Act has also reauthorized the Office of Substance Abuse Prevention (OSAP), and has elevated this office to institute level within ADAMHA. OSAP's responsibilities will include:

* supporting clinical training to substance abuse counselors and other health paraprofessionals involved in drug abuse education, prevention, and intervention;

* assisting communities in developing comprehensive long-term strategies for substance abuse prevention, and evaluating the success of different community approaches toward prevention; and

* supporting prevention, education, and treatment regarding drug and alcohol abuse relating to pregnant and postpartum women and their infants, as well as demonstration projects to provide drug treatment services to these women and infants.

ADAMHA will also be administering a one-time discretionary grant program to reduce waiting lists for drug abuse treatment; $75 million was appropriated for this program.

CLOSING REMARKS

As the focal point in the Federal government for research on reducing demand for illicit drugs, NIDA has a critical role to play in implementing the Anti-Drug Abuse Act of 1988. We are working with other components of ADAMHA and the Department of Health and Human Services, as well as with other Federal agencies, to implement the Act's provisions.

As I have noted, passage of the Act has given NIDA a 50 percent increase in our non-AIDS budget. This will allow us to capitalize on opportunities and expand our efforts in the priority areas of improving prevention and treatment strategies--including new medications for treatment, and more effective training for physicians and counselors involved in the drug abuse area-and of better understanding the 'effects of drug abuse on mothers and infants.

In addition, the $16 million that the Act has authorized for data collection will allow NIDA to reestablish data collection activities on the Nation's treatment programs as well as provide fuller and more frequent monitoring of the incidence and prevalence of illicit drug use. The data from these studies is essential for policy decisions in the treatment area.

We have recently established a Health Services Research Branch in our new Division of Applied Research. The $1.75 million provided by the Act will allow us to expand our research in the health services area. This research will be closely coordinated with our treatment evaluation activities, enabling us to provide more precise data on the cost effectiveness of various treatment interventions.

It is important to remember in any review of NIDA's research program that AIDS is the number one priority of the Public Health Service. Since drug abuse prevention and treatment is

also an AIDS prevention strategy, much of our attention will be directed toward the IV drug abusing population and their sexual partners and children.

The Anti-Drug Abuse Act has, in effect, opened wider the windows of opportunity in drug abuse research. NIDA will continue to seize research opportunities as they arise, but by providing continuity in our priority programs, we hope to encourage greater stability in important areas that demand research attention over several years. This Act-for the next three years, at least-has ensured that continuity.

In conclusion, I would like to comment on the interaction between NIDA and CPDD. I look at CPDD's annual meeting as a progress report for NIDA. I am very glad that Dr. Goodwin, the Director of ADAMHA, and Dr. Gordis, the Director of NIAAA, are here to see the breadth and depth of research being sponsored by NIDA. Ninety-one percent of the individuals presenting here are NIDA grantees. The quality of their research gives us all great hope that our understanding of the biological, behavioral, and social factors leading to drug experimentation-and, in some individuals, addiction-will lead to increasingly effective prevention and treatment interventions. The 1988 Anti-Drug Abuse Act has provided us with increased funds to continue our research efforts. We all know, however, that this increased funding must be sustained if we are to realize the gains which our current research promises.

AUTHOR:

Charles R. Schuster, Ph.D., Director
National Institute on Drug Abuse
5600 Fishers Lane
Rockville, MD 20857

Major Initiatives in Alcoholism Research: Current Questions, Future Answers

Enoch Gordis

INTRODUCTION

Until the early 1970s, alcohol abuse and alcoholism were not considered areas worthy of serious investigation. This situation changed dramatically with the creation of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) in 1971, as the focus for the Federal government's efforts to prevent and reduce the tremendous health, social, and economic consequences of alcohol abuse and alcoholism in the United States.

Over the years, the NIAAA mission has changed from its initial support for research and research training, clinical training, and prevention and treatment services, to its present one which focuses exclusively on research. We do not support prevention or treatment services, but provide the research evidence necessary to improve them. We also do not formulate or advocate for any particular public policies but seek to develop scientific information that can be used by the Congress and other policy makers and advocates as a part of their deliberations on various public policy measures.

Through an intramural scientific staff and an extensive program of extramural research grants and contracts, NIAAA supports epidemiologic research; research on the effects of alcohol on the human body; research on preventing and treating alcohol abuse and alcoholism; research training programs; health education development programs; a research-based services demonstration and evaluation program targeted at homeless persons with alcohol-related problems; and research on alcohol-related public policies that have broad implications for prevention, treatment, and rehabilitation.

Additionally, NIAAA maintains an active science communication program aimed at sharing with health care practitioners, policy makers, the general public, and others involved in managing alcohol-related programs about research findings with applicability to alcohol treatment and prevention efforts. Our scientific communications vehicles include publications such as Alcohol Health and Research World, a professional journal published quarterly; Alcohol Alert, a publication designed to

quickly disseminate research findings to alcohol clinicians; the special reports to Congress on alcohol and health, issued triennially; and specialized monographs and reports.

Research findings are also shared with the alcohol and general health care communities through two new on-line database services supported by the Institute. The first of these, the "Quick Facts" electronic bulletin board is available to anyone with a terminal and modem. Developed under the aegis of the Institute's Alcohol, Epidemiologic Data System (AEDS), Quick Facts provides access to alcohol-related epidemiologic data and facilitates communication among NIAAA staff and others interested in NIAAA programs and data. Scientists, clinicians, and others interested in alcohol-related research also have direct access to NIAAA's comprehensive bibliographic data bank, the "Alcohol and Alcohol Problems Science Database" through BRS, a commercial database vendor. The BRS title for the database is "EtOH," named after EtOH, one of the chemical designations for ethyl alcohol. ETCH covers literature from the late 1960's to the present, contains over 60,000 bibliographic records, and has grown to cover all aspects of alcohol research: psychology, psychiatry, physiology, biochemistry, epidemiology, sociology, neuroscience, treatment, prevention, education, accidents and safety, criminal justice, legislation, employment, labor and industry, and public policy. In addition to scientific and technical journals, the database contains entries on books, monographs, government reports, dissertations, and conference papers.

The Institute also collaborates with other national alcohol research institutions and is actively involved in a number of international collaborative studies.

This extensive agenda reflects the complexity of the problem NIAAA was created to address. Alcoholism and alcohol abuse have many roots and many manifestations; thus, the activities of NIAAA bring to bear the day-to-day energies of many people and a wide spectrum of organizations, joined in investigating, clarifying, and understanding one of the Nation's most troubling public health problems.

MAJOR RESEARCH INITIATIVES

Remarkable progress has been made over the past 15 years in understanding the causes and consequences of alcohol abuse and alcoholism. However, many basic questions remain concerning alcohol's action on the body, the mechanisms of dependence, alcohol's impact on individuals and groups in society: and alcohol's role in shaping the environment. For example, genetic research has contributed substantially to our understanding of alcoholism's etiology, however, we have not yet identified a genetic marker for a predisposition to alcoholism or developed a good understanding of the specific mixes of genetic/environmental factors that predispose individuals to alcoholism. The answer to one of the central questions in alcohol research-why people

continue, in the face of clear evidence of harm, to consume large quantities of alcohol. Another largely unanswered question is what factor or combination of factors are likely to be effective in preventing alcohol abuse and alcoholism in specific populations. We do not yet have scientifically valid evidence of the effectiveness of many of the commonly used treatments for alcoholism. This lack of evidence has major implication for continued public and private third-party support for alcoholism treatment.

Through alcohol research, we are asking these and other questions in our search to find way to help prevent and treat alcohol abuse and alcoholism. Some of the major activities in alcohol research that may provide answers to these perplexing questions are discussed below.

PREDISPOSING FACTORS

Genetic factors, environmental factors, and the interplay of genetics and the environment have all been implicated in the etiology of alcoholism. However, the precise nature of the specific genetic and environmental elements involved, and the complex processes by which they interact, have yet to be defined.

It has been known for many years that certain families have more than their share of alcoholic members, leading to suspicions that alcoholism may "run in families". Studies were initiated to define the biological and psychosocial factors that place some families, or specific individuals within these families, at greater risk for alcoholism. To distinguish the effect of learning from inheritance, investigators turned to the study of special populations such as twins, that permitted separation of these factors. Monozygotic (identical) twins who share all inherited traits were compared with dizygotic (fraternal) twins who share inherited traits in the same way as non-twin siblings. Adoptees who share an environment but not genes, and half-siblings who share only one biological parent who may or may not be the alcoholic, have also been studied. This research has provided evidence of a genetic component in defining the risk for alcoholism in a significant subset of the population. Currently, studies involving twins and adoptees are seeking to develop more refined knowledge about the genetic/environment interaction, as well as the genetic determinants.

It is clear that predisposing genetic factors may remain dormant in the absence of environmental cues and, further, that alcoholism may develop in the absence of genetic predisposition. It therefore has been important not only to identify the principal environmental factors, but also the manner and extent to which they interact with genetic factors. Consequently, another important area of research is the study of age-related patterns and predictors of alcohol use and abuse to identify environmental and psychological factors that may facilitate or constrain problem alcohol use. Under study as possible predictors

of later problem drinking are childhood antisocial behavior, parental drinking, cognitive functioning, attitudes, expectations, personality, environmental stress and coping, ethnicity, and cultural and socioeconomic factors.

Movement toward the development of an integrated causal explanation of alcoholism that takes into account biological, psychological, social, environmental, and developmental influences will be an important outcome of research during the next ten years. Formulating such an explanation will necessarily involve not only identifying factors that promote the development of alcoholism, but also factors that protect against it. The opportunity to develop non-invasive techniques for screening of high risk genotypes for alcoholism, and to employ knowledge about causal pathways in the prevention and treatment of alcoholism, will be important future developments. Over the next decade, the application of modern molecular genetic techniques to the study of alcohol-derived pathologic diseases should uncover the genetic factors contributing to these diseases, and could provide insights into the mechanisms by which these disorders develop. Applying the technique of restriction fragment length, polymorphism (RFLP) analysis in the study of a specific alcohol o derived pathology, such as in alcoholic pancreatitis or alcohol dementia, will provide an indication of whether a variant gene may be involved in the expression of the disease/ and provide information on its approximate chromosomal location and the nature of its inheritance pattern. Further research then may lead to the identification of the precise location of the gene on the chromosomal map, and then to the recognition of the affected gene and its protein product. This knowledge may be vital to uncovering the mechanism by which injury is produced and lead to preventive and therapeutic remediation.

To further advance this immensely important area NIAAA has developed a collaborative research program in genetics. Involving both NIAAA intramural scientists, and extramural scientists, this activity will begin to examine the heritage nature of traits associated with the risk of alcoholism and apply molecular genetic studies to begin mapping genes important in alcoholism, thereby providing valuable new information about the inheritance patterns of alcoholism.

ALCOHOL AND DEPENDENCE

The majority of adult Americans who consume alcoholic beverages do so without problems. A second group of problem drinkers or alcohol abusers experience negative consequences of their drinking (e.g., accidental injury and death, the loss of spouse, children, employment, etc.) but are not dependent on alcohol. For these individuals, health, social,. and economic consequences develop secondary to alcohol use because of poor judgment, failure to understand the risks, or lack of concern about the damage to themselves or others. A third group, however, have the disease called alcoholism and are dependent on the drug alcohol.

These drinkers became ill when drinking is interrupted (i.e., they experience an abstinence or withdrawal syndrome); they are tolerant to large amounts of alcohol; they miss alcohol intently or "crave" alcohol at times when they are abstinent; and, in the real world, they frequently display lack of control over their drinking. Unlike normal drinkers who seem to have an internal signal which says "enough", alcoholics generally cannot control the amount of alcohol they drink on any occasion. They drink compulsively despite the obvious damage their drinking causes. Why is this so? Research to answer this question, particularly research on alcohol's effect on the brain, has been progressing at an unprecedented pace.

Though alcohol undoubtedly interacts with and affects virtually all organs, the expression of alcohol's action on the brain is of particular importance. Alcohol's actions include not only intoxication, but the additional phenomena of tolerance and dependence. Tolerance is the ability of the brain to adapt to the presence of alcohol such that greater and greater quantities of alcohol become necessary to produce the same effects. This increases the likelihood that particular individuals drinking for some desired effect or feeling will increase their alcohol consumption. Dependence is the adaptation in structure and function of the nervous system as a consequence of chronic alcohol use, which makes it necessary for the drinker to continue consuming alcohol in order to prevent the unpleasant withdrawal reaction.

An abundance of information derived from basic research in brain morphology, chemistry, and electrophysiology has provided a strong background for asking focused questions concerning the effects of alcohol on the brain. Proceeding from current findings and using state-of-the-art neuroscience and biobehavioral techniques, investigators hope to illuminate the mechanisms underlying alcohol intoxication, tolerance, dependence, reinforcement, and craving inherent to alcohol abuse and to the disease of alcoholism.

For instance, although it is well known that alcohol exerts profound effects on brain function, the cellular and biochemical bases for its actions are not yet clear. In efforts to explore the processes of intoxication, tolerance, and dependence, current research is characterizing the role of specific neurotransmitter systems; the effects of alcohol on nerve cell membranes; determinants of alcohol craving; and the structural and functional changes seen in chronic alcohol use. Progress made in understanding the acute and chronic effects of alcohol in the central nervous system has been encouraging and is already providing direction for the development of new therapeutic agents.

Intoxication, tolerance, and dependence have been studied at a number of functional levels within the brain, including that of the individual brain cell, or neuron. Each neuron is surrounded by a membrane that is composed principally of fat-like molecules

called lipids, and of proteins embedded in the lipid structure. It now appears that a significant degree of alcohol's actions on the brain are exerted through the interactions of the alcohol molecule with lipids and proteins of the cell membrane. The disruption of the cell membrane may account substantially for the phenomena of intoxication, and the adaptations of the cell membrane to the presence of alcohol may account substantially for many facets of tolerance and dependence.

Neuroscience research is helping to lay the groundwork for understanding why alcohol consumption is so rewarding to some people-that i they continue to drink even after they develop severe health, social, and economic problems. Scientists hope to identify the neuronal systems in the brain which determine the pleasurable effects produced by alcohol and to learn if these sites in the brain account for the pleasure produced by other abused substances, such as cocaine and heroin.

There are many chemical messengers or neurotransmitters that allow the neurons of the brain to communicate with each other. One neurotransmitter that now appears to have particular importance for explaining alcohol's action is gamma-aminobutyric acid (GABA). A number of drugs with actions similar to alcohol also appear to affect the GABA neurotransmitter system, including the barbiturates and the benzodiazepine tranquilizers, such as Valium. Some of the more interesting recent findings concern the actions of new drugs that, while chemically similar to Valium, have an effect opposite to that of a tranquilizer. These new drugs have been found to block alcohol's action on the GABA system in the brain and to reverse some of alcohol's effects on behavior, such as loss of motor coordination.

During the next ten years it may be possible for neuroscientists to determine the physiological processes that mediate the relationship between alcohol craving and the appetitive behaviors of hunger and thirst. Understanding the basic human appetitive process could be the critical determinant for developing successful alcoholism treatment programs. Insight into the neurochemical actions of alcohol on neurotransmitter and membrane receptor systems should also be forthcoming.

Most exciting in the coming years is the very real possibility of using individual brain imaging techniques to identify neural substrates inherent to alcohol-related phenomena, but to combine information derived from different techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), and electrophysiological studies to create new conceptions of the dynamic workings of the brain. At the very least, integrating data from studies that have applied different technologies will help resolve uncertainties about particular brain pathways and behaviors that regulate responses to alcohol. Computer-aided modeling of cell assemblies will be an additional innovation. Because of the complex pharmacological effects of alcohol and the sheer number of brain regions affected by alcohol, it is

generally recognized that a global understanding of alcohol's actions will require sophisticated technological models of networks of brain activity- In the 1990 's, brain and behavioral science research will also be in a position to incorporate computer-aided modeling and imaging techniques that will allow for a better understanding of addictive behavior and enable early detection of alcohol -related problems.

PREVENTING ALOOHOL-RELATED PROBLEMS

Prevention is one of the most difficult area of research with which our Institute has to deal - difficult because of the need to study real-life situations outside of the laboratory and the resulting number of variables beyond the researcher's control. Yet, it is vitally important that we determine what strategies are most effective in preventing alcohol and other drug problems among our Nation's many subpopulation groups, particularly given the current national concern for preventing alcohol and other drug abuse and the massive public and private expenditures for prevention-related activities.

In 1987, NIAAA gave added prominence to prevention research by establishing a Prevention Research Branch within a new extramural Division of Clinical and Prevention Research. Areas of current research interest focus on preventing alcohol abuse by encouraging changes in an individual or groups of high-risk individuals; changes in the environment; and examinations of the complex, synergistic relationships between the actions of individuals, groups and the larger social system.

In planning prevention research in the alcohol arena, it is important that investigators identify the outcome or the relevant drinking behavior to be prevented (e.g. , addiction, sequelae of alcoholism such as cirrhosis, injurious consequences of alcohol abuse such as traffic accidents, violence and absenteeism, etc.) . Regardless of the choice of outcome measure, alcohol prevention research has common underlying themes: A systematic quest for effective strategies to reduce the incidence and prevalence of alcohol abuse and alcoholism; and/or a systematic quest for mechanisms to ensure the diffusion and adoption of prevention strategies proven to be effective.

The questions that remain unanswered relative to our ability to prevent alcohol abuse and alcoholism are many. For example, with respect to youth and young adults, what are the effects of peer pressure, decision making skills, and personality variables on the drinking habits of high school and college students? What are the effects of expectancies and sociocultural variables on early drinking habits, as well as changes in the drinking patterns of youth over time. Because of the importance of the early years on development throughout the life span, the efficacy of intervention efforts for young children of alcoholics and the impact of parenting skills training also are important areas for research investigation.

Another prime area for research on effective prevention technologies is that of preventing alcohol-related problems among special population groups. Business and professional women, Mexican-American women, urban American Indians, and the elderly are among the special populations currently under study. Some researchers are investigating whether these populations have unique qualities that may put certain members at high risk for alcohol abuse. Others are testing the efficacy of specially designed prevention strategies for members of these target groups.

Scientific inquiry of the effectiveness of environmental change in reducing alcohol-related problems is another vital component of prevention research. An example of this type of research is to determine whether the incidence of teenage drinking and driving can be reduced by changing the minimum drinking age or the price of alcoholic beverages. The involvement of alcohol in motor vehicle accidents and in crime is also being examined. Future research topics include the impact of changes in alcohol beverage control (ABC) laws on problem drinking; the impact of differences in the availability of alcoholic beverages on problem drinking; the effects of informal drinking groups on the consumption behavior of participants; and the influence of life stressors, social resources, and coping responses on remission and relapse among alcohol abusers.

Preventing alcohol-related problems through the workplace is another area of prevention research emphasis. Employee assistance programs (EAPS) have grown and expanded dramatically over the past decade. Studies are being conducted to determine the extent to which the work environment may improve or worsen problem drinking and the comparative effectiveness of locating intervention programs within or outside the work setting. The structural characteristics of the worksite and the unique characteristics of employees are being examined for their complementary contribution to preventing relapse of alcohol problems. Additionally, the degree to which the attitudes of key worksite personnel influence EAPs is under study.

Over the next 10 years, efforts will be aimed at applying the rapidly expanding knowledge on both alcohol abuse and on overall disease prevention strategies to devise and evaluate new prevention approaches. Contributing to the development of these approaches will be knowledge from current research on factors implicated in the initiation and maintenance of alcohol abuse, including peer and parental influence and risk-taking behavior. Advances from research on genetics in behavioral predictors will allow for the development of more highly focused prevention strategies. The decade also will afford the opportunity to apply successful methods of community intervention, derived from studies directed at reducing risks for heart disease and hypertension, to prevention of alcohol abuse. The field of prevention research will also benefit from the application of

innovative statistical techniques and new behavioral research methods permitting the rapid assessment of the impact of intervention approaches and public policy.

TREATING ALCOHOL ABUSE AND ALCOHOLISM

Treatment outcome research and the analysis of treatment outcome are fundamental to our ability to treat alcohol abusers and alcoholics. It is clear that the alcohol field can no longer rely on intuition and experience alone to justify* treatment; the Congress, third-party payors, the public, and the alcoholic will require that the effectiveness of alcoholism treatments be supported by the same evidentiary base of scientific information that is required for all the treatments for all other diseases. Thus, research on the efficacy of currently practiced treatment strategies and on developing new therapies, continues to be a major NIAAA priority. As with prevention research, the Institute has elevated treatment research within the organization to emphasize its importance.

Primary attention paid to answering the question, what type of treatment will be most effective in treating which type of patient. Advances are being made in differentiating the typologies of alcoholics and in developing strategies to match differing patients to appropriate therapies. For example, several studies suggest that individuals who have strong social support system and who do not suffer major psychiatric problems may do as well in outpatient programs as in more intensive inpatient settings. The effectiveness of specific therapies for alcoholism treatment also are under study, including family therapy, relaps prevention, and behavioral approaches, such as community reinforcement. At the end of this year, a major multi-site cooperative study will be undertaken, this study, which involves clinical trials of strategies for matching particular patients to the most suitable treatment approach, will have important implications for improving the effectiveness of alcoholism treatment.

Another important component of alcoholism treatment research is the development and assessment of pharmacological agents which may eventually aid in the treatment process. Thus far, there are few drugs available that are useful in the treatment of alcoholism. However, recent studies from the field of psychopharmacology have shown that certain pharmacologic agents, such as compounds that block the re-uptake of the neurotransmitter serotonin, and anxiolytic drugs that lack the abuse potential of the benzodiazepines, have actions that may be useful as adjuncts to the standard alcoholism treatment. Research is ongoing to test whether, and for whom, such drugs may be useful.

Effective treatment of alcoholism over the next decade will benefit from rigorous studies evaluating therapies treatment professionals are currently using. Studies will explore differing

factors that may relate to the efficacy or particular treatment approaches, such as a patient's background or personality, characteristics of the therapist, and the treatment setting. Such research will enhance our ability to match patients with the most effective treatment to suit their needs.

During the next decade, investigators will be able to examine the effectiveness of new pharmacological approaches that offer potential benefits for alcoholism treatment research. For example, drugs that interfere with the reward properties of alcohol may be found to block the phenomenon of craving and become a valuable adjunct to alcoholism treatment.

With a more precise understanding of the basis of the physiology of alcohol withdrawal, new drugs may be developed that allow improved medical management of the withdrawal syndrome, or reduce the potential of delirium tremens. Research also may provide us with a better understanding of how the withdrawal experience affects both subsequent drinking and the potential for effective treatment.

Due to the magnitude of health problems resulting from alcohol abuse and alcoholism, there is growing need to improve methods of diagnosing alcohol-related problems. The development of objective markers of alcohol consumption would be one such means to permit a physician to easily assess a patient's history of alcohol intake.

Significant inroads in this direction have been made by NIAAA intramural research staff who initiated studies on easily accessible blood cells and examined the enzymes adenylate cyclase and monoamine oxidase which they had previously demonstrated in their studies with animals. Human alcoholics were found to be significantly different from control subjects in these enzyme activities, and more than 75 percent of the alcoholics could be identified using the test of these enzyme activities. An interesting feature of this study was the finding that the differences between alcoholics and controls in enzyme activity was evident for long periods, even if the individual remained sober. This observation indicated that the observed difference may be an inherent or inherited characteristics of alcoholic subjects. Further extensive work is necessary to determine whether the findings are indications of "state" or "trait" differences between alcoholics and controls. The work, however, provides some of the first indications that there may be reliable markers not only for measuring an individual's prior history of alcohol intake, but for measuring genetic predisposition in certain individuals to develop alcohol-related problems during their lifetime, even if they are not experiencing such problems. Such diagnostic tools would provide for the physician, or other treatment specialist, means for appropriate identification and classification of patients for more specific treatment and prevention efforts.

The development of objective markers of alcohol consumption also will have a significant impact on alcoholism treatment research. The decade offers the potential for developing objective marker technologies in kit form. The kits would provide alcoholism researchers, as well as clinicians, the opportunity to monitor treatment compliance independent of sometimes unreliable patient self-reports. Such objective markers have the potential, thereby, for significantly enhancing treatment efficacy. The prospect for developing genotypic and phenotypic markers of alcohol dependence may further contribute to the identification of patients with particular treatment needs, and further enhance the potential for treatment effectiveness.

CONCLUSION

Each new discovery made by alcohol researchers-however remote it may seem to the ongoing business of helping alcohol abusers-provides a piece of the answer to the ages-long question of what is alcohol abuse and alcoholism and how can it be prevented and treated. We see the future both as a challenge and as a reward: A challenge because with more answers come more questions and we still have far to go. A reward, because of the knowledge that the answers we find ultimately will help diminish a public health threat that has existed for far too long.

* Enoch Gordis, M.D., Director
Federal National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland.

Introduction of Nathan B. Eddy Memorial Award

Louis S. Harris

It is a great pleasure and honor to be asked to introduce Dr. Leo E. Hollister, the recipient of this year's Nathan B. Eddy Award. Dr. Hollister is a distinguished clinical pharmacologist who has made and continues to make important contributions to drug abuse research and the Committee on Problems of Drug Dependence.

Dr. Hollister was born and educated in Cincinnati, culminating with an M.D. degree from the University of Cincinnati in 1943. After an internship at the Boston City Hospital and residency at Cincinnati, he was on active duty with the U.S. Naval Reserve from 1945-46, a task he took on again in 1950-51. He joined the Veterans Administration System (V.A.) in San Francisco in 1947, an association he maintained until his retirement in 1986. Along with his V.A. appointment, Dr. Hollister always maintained an academic appointment; first at the University of California, San Francisco and later at Stanford University where he rose through the ranks to Professor of Medicine, Psychiatry and Pharmacology. These multiple academic appointments provide a strong indication of Dr. Hollister's broad interests and expertise. On retirement from the V.A. and Stanford, Dr. Hollister has taken on a new position as Medical Director, Harris County Psychiatric Center in Houston, Texas and Professor of Psychiatry and Pharmacology at the University of Texas at Houston.

As a clinical pharmacologist, Dr. Hollister has made numerous contributions to the development of new psychotherapeutic agents and to our understanding of the action of psychotropic drugs. His extensive publications are a wonderful demonstration of what a clinical pharmacologist should, in the best sense, be. In recognition of his contributions, Dr. Hollister has been awarded the Meritorious Service Award and the William S. Middleton Award for Outstanding Achievement in Medical Research from the V.A. He has also received the Taylor Manor Award for Outstanding Service to the American College of Neuropsychopharmacology (ACNP), the William Menninger Memorial Award from the American College of Physicians and the Oscar B. Hunter Award for Experimental Therapeutics from the American Society of Clinical Pharmacology and Therapeutics (ASCPT).

Dr. Hollister is a dedicated member of many professional societies and advisory boards. For instance, he has been President of ASCPT, ACNP and CINP. He chaired the Committee on Problems of Drug Dependence from 1972-77, and served as Executive Secretary from 1979-81, a period which saw the Committee move from the National Academy of Sciences to its present status as a respected

independent body. Without his dedicated leadership, I doubt this would have been accomplished.

Thus, with the citation:

“Leo E. Hollister, brilliant clinical pharmacologist, dedicated public servant, the Committee on Problems of Drug Dependence salutes you with the Nathan B. Eddy Memorial Award.”

AFFILATION:
Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613

Peregrinations Among Drugs of Dependence: Nathan B. Eddy Memorial Award Lecture

Leo E. Hollister

It is a special pleasure to receive this award which memorializes the contributions of Nathan B. Eddy to the field of drug dependence. I was privileged to know Dr. Eddy during the last several years of his life. It has also been my privilege, through a long association with the Committee on Problems of Drug Dependence, to have known personally all previous winners of this award. There also seems to be a good possibility that through that association I shall know many future winners of this award.

My entry into the field of drug dependence, as with other aspects of my career, was somewhat fortuitous. Studies in the laboratory of Dr. Sidney Raffel, Professor of Microbiology at Stanford, had shown the chlorpromazine had static action against M. tuberculosis in concentrations of 5 ug/ml and cidal action in concentrations of 10 ug/ml. As there were not too many drugs available in the 1950s effective against this mycobacterium, we organized a trial of chlorpromazine in patients with pulmonary tuberculosis.

Thirty six patients entered a blind, controlled trial, 17 of whom received chlorpromazine in daily doses of 300 mg with the majority receiving such doses of 6 months or more. Nineteen other patients were assigned randomly to placebo. We found little difference between the two groups in terms of infectiousness of sputum or x-ray changes. When the study was terminated, the drugs were stopped abruptly. Four of the 17 patients on chlorpromazine developed immediate symptoms of restlessness, insomnia and gastrointestinal disturbance which suggested a withdrawal syndrome. These responded promptly to resuming chlorpromazine or use of sedatives. None of the 19 patients withdrawn from placebo showed such effects.

This experience was mentioned in a few lines in a paper published in the American Review of Respiratory Disease,

hardly a widely read journal in the field of drug dependence. I was still naive enough to believe that once you had mentioned a phenomenon in the medical literature, there was no need to repeat such mention. In retrospect, the experience was notable. First, it was probably the first placebo-controlled demonstration of a drug withdrawal syndrome. Second, it was probably the first documentation of a withdrawal syndrome in a class of drugs other than those known to be abused. Third, it was probably the first experience of a withdrawal syndrome occurring in the context of therapeutic doses. Talk about missing the boat!

I had become aware of the superb work being done at the Addiction Research Center at Lexington in explicating the mechanism of withdrawal to alcohol and barbiturates. Having studied the newest sedative, meprobamate, and having seen some instances of spontaneous withdrawal from that drug, I decided to borrow the Lexington techniques to study the phenomenon experimentally. We accomplished three studies. The first substituted placebo for meprobamate given chronically in doses thought to be within the therapeutic range (median 1600 mg/day). Only 10 of 60 patients abruptly withdrawn by placebo substitution experimented mild reactions, usually those who had received higher than average doses. We concluded that "symptoms from withdrawal at ordinary therapeutic doses were infrequent and mild, resembling recrudescence of symptoms being treated". That statement pretty well summarizes the story of therapeutic-dose dependence today. Our second study looked at the results of placebo substitution in patients (largely schizophrenics for whom the drug was being tried to determine whether it might be helpful), who had been treated chronically with "supra-therapeutic" doses (3.2 to 8 g/day). Sixteen of 21 patients experienced a classical sedative withdrawal syndrome soon after placebo substitution; 14 of 19 tested showed abnormal EEGs which later reverted to normal. However, no seizures were observed. In a third study, 8 of 10 patients treated with similar doses of meprobamate in combination with the phenothiazine, promazine, also developed definite withdrawal, with a seizure in one patient at 72 hours. Promazine, rather than protecting against withdrawal from meprobamate, probably aggravated it. These studies emulated those of the Lexington group - not a bad example to follow.

During the course of these studies, we measured plasma concentrations of meprobamate. The plasma half-life following chronic administration was estimated to be 24 hours, with the usual wide interindividual variations. Later, we found the plasma half-life following single doses to be a median of 11 hours. The time-course of withdrawal reactions we observed with meprobamate was similar to that which had been described by the Lexington group for short-acting barbiturates.

During 1959, I was invited to attend a pre-marketing meeting for the new anxiolytic, chlordiazepoxide, even though I had not studied the drug myself. At the conclusion of that meeting, after hearing the way the drug was extolled, I thought that if it were half as good as touted, it would very likely be abused. This led me to initiate a study in which the drug was used in high doses in schizophrenic patients (to determine possible efficacy) but also to provide a basis for a discontinuation trial. Eleven patients were treated with daily doses of 300-600 mg for a median period of 5-6 months. Under careful observation, they were abruptly switched to placebos. Ten of the 11 patients demonstrated withdrawal manifestations, although onset was delayed until the second or third day and the peak was not attained for a couple of days more. Two patients developed seizures, at 7 and 8 days post-withdrawal. Measurement of plasma concentrations of the drug, and by the methods then available probably all its metabolites, indicated a possible half-life of 48 hours, which accounted for the attenuated time-course of the withdrawal syndrome. This experiment was published prior to the introduction of chlordiazepoxide into medical practice. In a subsequent study of the effects of diazepam in schizophrenics, one of the participating hospitals (without my knowledge or assent) abruptly discontinued patients from doses of 120 mg/day and demonstrated a similar attenuated withdrawal reaction. This study, too, was published prior to release of diazepam into clinical practice. Somewhat to my disappointment, it took several years before spontaneous instances of abuse of these benzodiazepines and withdrawal reactions were reported in the clinical literature. Nature took a long time to imitate art.

The meprobamate homolog, tybamate, provided an opportunity to observe a different aspect of sedative drug withdrawal. Doses of 4.9 to 14 g/day given for 4 to 16 weeks and then abruptly withdrawn failed to elicit a withdrawal reaction. The reason was that the plasma half-life of this drug is so short, from 2-3 hours, that it was impossible to sustain continued high concentrations with ordinary dosage schedules. This experience, added to our others and to clinical experience, led to the postulation that the rapidity of onset and severity of a sedative drug withdrawal syndrome is a function of the plasma half-life of the drug. Drugs having half-lives of 6 to 24 hours are associated with the most severe withdrawal; drugs, such as tybamate, with very short half-lives, are unlikely to produce withdrawal; drugs with long half-lives, such as chlordiazepoxide and diazepam, produce attenuated withdrawal reactions as we bad first noted; and drugs with very long half-lives, such as phenobarbital, generally produce no discernible withdrawal syndrome. This schema has stood the test of time and is appropriate even today,

By the late 1950s, some generally poor work with lysergic acid diethylamide (LSD) had been published. I felt I could do better. Drugs of this type were easily obtained, LSD from a friend who worked for Sandoz and mescaline by mail from Nutritional Biochemicals Corporation. Subsequently, I became engaged in a series of studies of these and other hallucinogens that developed meticulous descriptions of their clinical syndromes and time course, quantified responses to different doses, tested a variety of psychometric and psychophysical effects, and measured a variety of biochemical parameters.

Early on, we found that LSD and mescaline (as well as the newly discovered hallucinogen, psilocybin) produced virtually identical clinical syndromes despite doses differing by orders of magnitude. The concept of the LSD group of drugs was born, as contrasted with anticholinergic hallucinogens (the JB series of compounds named after John Biehl, the chemist who synthesized them) or drugs such as alpha- methyltryptamine, which had both amphetaminelike and LSD-like effects.

To obtain information about the time-course of clinical effects, we placed a tape recorder in the room with the subject. I would enter and after providing a time cue would engage the subject in describing their experiences. It was my job each evening to review the tapes to reduce this material to data. One of my early subjects was a creative writing student at Stanford who said he was writing a novel and wanted to take these drugs to see what it was like to be crazy. After warning him that these drug effects were not especially like those of schizophrenia, he enlisted in one of our studies. When I entered his room to stimulate discussion, there was no need; he was already expanding to the recorder his experiences. #en I later listened to these tapes, it was obvious that this chap had enormous talent; most of what he described was unique and probably fictional, but the descriptions showed a masterly command of language. When “One Flew Over the Cuckoo’s Nest” was published, I thought it possible that hallucinogens had indeed unleashed some creativity. Later, I came to an opposite conclusion. Ken Kesey may have been the subject most harmed by our experiments with these drugs.

Our studies demonstrated a remarkable similarity between members of the LSD group, not only in clinical syndromes but in most other aspects. The impairments they produced in psychometric tests were mainly due to inability to sustain attention; they enhanced aspects of color perception, including subjective colors; they had similar effects of enhancing primary suggestibility. Their sympathomimetic actions caused profound elevations of plasma free fatty acids and significant reductions in serum phosphorus levels.

Several assertions were made that, if true, would have made hallucinogenic drugs highly useful pharmacological tools or treatments. First, these drugs were alleged to provide a "model psychosis", similar to that of schizophrenia. Presumably by studying their mechanisms of action, we might learn something about the pathogenesis of schizophrenia. To test the model psychosis notion, I took tapes of subjects under the influence of hallucinogens as well as patients with schizophrenia and carefully edited out any allusions that might betray their origin. Nonetheless, it was easy for a variety of mental health professionals to identify correctly which tape came from which source. The psychotomimetics did not really mimic schizophrenia. This experience, as well as one with a potential endogenous psychotogen, 3,4-dimethoxyphenylethylamine, found in the urine of schizophrenics, led to a series of postulates that such a substance would have to meet. To date, no proposed endogenous psychotogen has met these criteria.

A second claim was that LSD, in large single doses, could provide enough insight to cure alcoholics. We compared a dose of 600 ug of LSD with a dose of 60 mg of dextroamphetamine, having devised a new rating instrument, the Drinking Behavior Inventory, to grade degrees of alcoholism. A slight advantage was found for LSD at 3 months followup but by 6 months, both treatments were equal. Unknown to me at the time, three other groups were also testing this therapeutic use. Although each group used different techniques, the conclusions were identical. LSD was not an effective treatment for alcoholism.

A third claim was that psychedelics, as they were then being called, would facilitate psychotherapy by breaking down resistances. We took patients with various disorders who had plateaued in psychotherapy and made recordings of their psychotherapeutic interviews during 5 conditions: no drug, placebo, LSD, mescaline and psilocybin. These were then rated blindly by an experienced psychotherapist for the meaningful content of each session. Mescaline was slightly better than the other treatments in providing increased introspection, but none of the conditions was significantly different from the other. One might have loosened up the patients better with two martinis.

My first meeting with Nathan Eddy occurred during the mid-1960s. The Veterans Administration had been enlisted for a series of cooperative trials evaluating new analgesics, under the prodding of Dr. Eddy and the CPDD. The chief investigator was to be Bill Forrest, who was based at my hospital. As I was then in charge of research there, it must have seemed desirable to get my blessing. So I was summoned to a meeting where every person was already known to me except Eddy.

Talk about presence with a capital P. I met a tall thin man with a myasthenic face, eyes covered with bottle-bottom lenses, with another set of lenses above them as an eye-shade. Who could not have been impressed? He didn't say much, but when he did it was in crisp language that was right to the point. The great man more than lived up to his billing. After the meeting, I had little idea that our paths would later join.

During a committee meeting in Washington in June, 1967, I happened to run into Milton Joffe, then with the FDA. He mentioned that a new drug, given the epithet, "STP", had appeared on the streets of San Francisco. His agency would like to know more about it. They had already signed a contract to study the drug with a group at Johns Hopkins headed by a young fellow named Sol Snyder. I told Milton that I had a protocol for LSD in my office file that could easily be adapted to 2,5-dimethoxy-4-methylamphetamine. I would study the drug without a contract. He went to his unlocked desk and gave me an envelope with an ample amount. I went from his office to catch the Friday evening plane to San Francisco. By the following Tuesday our first STP subject was being studied. How greatly things have changed! In a very short time, we proved that STP belonged in the LSD group, was more potent than mescaline, that tolerance to it quickly developed, and that its effects could be partially mitigated by chlorpromazine. By early September, I sent a report to Joffe who passed it on to the members of the CPDD, including Nathan Eddy, who had instigated the inquiry. Not long after, I was approached about becoming a member of the CPDD, the beginning of a long association. I have little doubt that Eddy himself suggested my recruitment.

That same summer, new ways of studying an old drug became available. The forerunner of NIDA, under the guidance of Sidney Cohen, had arranged to supply various marijuana components, including tetrahydrocannabinol (THC), to investigators. I thought it would be of some interest to compare authentic THC with synhexyl, a synthetic homolog which had been studied in the 1940s, to determine the extent to which the earlier studies might be pertinent to marijuana. Through R. K. Richards, a pharmacologist recently retired from Abbott, I was able to obtain some 25-year old synhexyl, a brownish-black spot of tar in a small vial. We quickly defined the clinical syndrome from these two drugs, which was similar, the time course, which was somewhat delayed for synhexyl, and the fact that synhexyl was less potent. As it turned out, the Lexington group, headed by Harris Isbell, scooped us on THC, but our studies were complementary.

Thus, began a series of studies of THC and its homologs which has continued to the present. From the very first, we

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were interested in structure-activity relationships and systematically explored the relative potency of various cannabinoids and THC metabolites for THC-like activity. The two major cannabinoids in marijuana other than THC, cannabidiol and cannabinol, had no THC-like activity. Of all cannabinoids, THC had the greatest activity and of all THC metabolites, only one, 11-hydroxy-THC, had as much, or according to our estimate, slightly more activity.

Our studies of cannabinoids over the past 22 years have touched upon virtually every aspect of their actions. They constitute the largest series of studies of the human pharmacology of marijuana on record. Some have been concerned with drug interactions, most notably with other cannabinoids; we found none. Others have been concerned with psychological functions, such as various aspects of memory, time sense, goal-directed behavior, and expectancy (contingent negative variation). Motor functions studied have been effects on driving and the roadside sobriety test, as well as residual impairment in simulated airplane operation. Physiologic effects, such as those on appetite and hunger, glucose tolerance, electrocardiogram and sleep have been looked at. A variety of other studies have examined the relationship between marijuana use and aggravation of schizophrenia, the influence of setting on the marijuana experience, and possible cytogenetic alterations from the drug. As most of these studies have been fairly recent and may be familiar, they will not be described in detail.

At some point in studying drugs, it becomes desirable to measure plasma concentrations to obtain a sense of the drug's pharmacokinetics and to try to relate these to clinical effects. The Swedish group, headed by Stig Agurell, was so far ahead in techniques of measurement that I despaired of ever being able to catch them. So following the old saying, "If you can't lick them, join them" we have collaborated with them during the past 10 years, the clinical work being done in the US and the laboratory measurements being done in Sweden. This cooperation has elucidated the patterns of drug availability from three routes of administration (smoking, ingestion and intravenous administration), has found that the kinetics of the inactive cannabinoids, CBD and CBN are similar to those of THC, has found that heavy marijuana use as compared with light use does not much change the kinetics, and has correlated clinical actions with plasma concentrations. A number of Swedish PhD candidates have based their theses on this work.

I shall not mention my modest endeavors in the area of alcohol use and opiate dependence. These are dwarfed by those of many others who have made much more extensive studies. still, the whole phenomenon of non-medical use of drugs

commands attention. Enough needs to be done to require the lifetimes of all of us in this room, and then some.

Please accept my thanks for this great honor, which I shall surely cherish. Even more, I shall cherish the memory of the many able scientists and wonderful people that my long association with the CPDD has provided. Thank you all.

AUTHOR:

Leo E. Hollister, M.D.
University of Texas Medical School
at Houston
Houston, Texas

Contamination of Clandestinely Prepared Drugs With Synthetic By-Products

William H. Soine

On the basis of the number of clandestine laboratories seized by the Drug Enforcement Administration (DEA), the use of clandestinely synthesized drugs would appear to be increasing. The number of laboratories closed by the DEA has increased from 184 laboratories in fiscal year 1981 to 647 laboratories in fiscal year 1987. Although many risks are associated with the abuse of drugs, purity is a major danger associated with these clandestinely prepared drugs (Soine 1986). Compounds which can affect drug purity can be classified as diluents, adulterants, impurities of manufacture and impurities of origin. The presence of diluents and adulterants is highly variable, however, this is not true for the impurities associated with the synthetic process. The impurities of manufacture and origin are usually present and will vary primarily in concentration. Therefore, it is not surprising that these impurities may contribute to the pharmacological or toxicological effects associated with clandestinely synthesized drugs.

This report updates information that was published in a prior review on this subject (Soine 1986). The drugs discussed are limited to the stimulants, amphetamines and cocaine. The synthetic method for each drug is discussed, followed by the identification and occurence of synthetic impurities and their associated pharmacology/toxicology.

AMPHETAMINES

There are numerous methods reported for the synthesis of amphetamine -(Frank 1983), as shown in Figure 1. The major methods utilized by the clandestine chemists use starting materials that can be easily purchased and/or synthesized. The major amphetamine synthesized in the United States is methamphetnmine, whereas in Europe, it appears to be amphetamine. An important precursor for the synthesis of both amphetamine and methamphetamine is phenyl-2-propanone (P2P). Four basic synthetic routes are used for the synthesis of P2P (Frank 1983). The most frequently used method starts with phenylacetic acid, acetic anhydride and sodium acetate or pyridine. It is this reason that sales of large quantities of phenylacetic acid (1 kg) are monitored by the DEA. The most commonly observed by-product present in P2P prepared by this

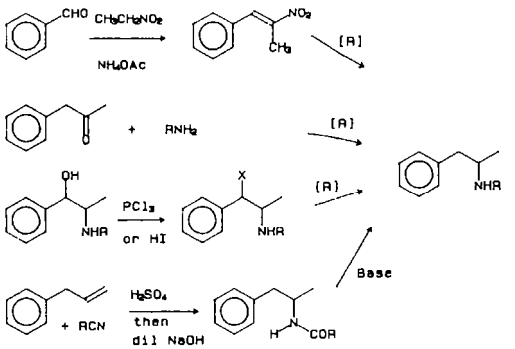

Figure 1

method is dibenzylketone (Herbst and Manske 1943). Dibenzylketone will produce a synthetic impurity via the same reductive amination reactions as P2P to give 2-(phenylmethyl)phenethylamine (or the N-methyl homolog in the case of methamphetamine). The methods using allylbenzene or ß-methylstyrene (Dal Cason et al., 1984) are analogous to the methods used clandestinely for the synthesis of 3,4-methylenedioxyphenyl-2-propanone (to be used in the synthesis of 3,4-methylenedioxyamphetamines) from safrole or isosafrole (Ellern 1985, Hanson 1988). Impurities present in P2P using allylbenzene or ß-methylsytrene have not been reported.

The Leuckart method is still frequently encountered as a method for synthesis of both amphetamine and methamphetamine. Until 1981, the main impurity present in amphetamine clandestinely prepared in the Netherlands was 4-methyl-5-phenylpyrimidine (Huizer et al., 1985). After 1981 di-(ß-phenylisopropyl)amines, DPIA, (or the N-formyl derivative, or both) were the main impurities observed in amphetamine in both the Netherlands and in Norway (Lambrechts et al., 1986). It was hypothesized that the change in impurity pattern was due to the addition of formic acid during the P2P and formamide condensation step. It should be noted that DPIA isomers have two chiral centers, therefore both a diastereomeric pair and a meso stereoisomer are usually present in illicit amphetamines prepared via the Leuckart method.

The most popular method for the synthesis of methamphetamine is via reductive amination using P2P, methylamine, aluminum foil, mercuric chloride (catalytic amount) diethyl ether and isopropano1 (Frank 1983). This reaction goes in very high yields and a very pure product can be obtained. It has been observed that samples of illicit methamphetamine from numerous clandestine laboratories have been found to contain mercury ranging from trace levels to 1300 ppm mercury (Davidson 1983). Additional basic contaminants have not been reported for this synthetic route.

The second most frequently encountered synthesis of methamphetamine is from ephedrines by conversion to the chloroephedrines followed by reduction (Allen and Kiser 1987, Cantrell et al.,

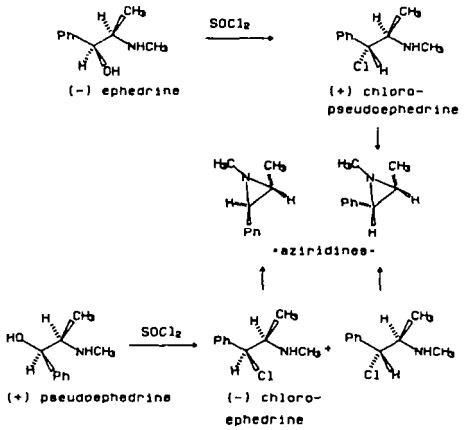

Figure 2

1988). (-)-Ephedrine or (+)-pseudoephedrine is converted to (+)-chloropseudoephedrine or (-)-chloroephedrine, respectively, using $SOC1_2$, $PC1_3$, $PC1_5$ or $POC1_3$. The reductive dehalogenation to methamphetamine is accomplished using H_2/catalyst, HI or HI and red phosphorous (DEA Dec. 1986). The synthetic impurities present in samples using this method are (+)-chloropseudoephedrine or (-)-chloroephedrine which also cyclize to form cis- or trans-1,2-dimethyl-3-phenylaziridines, (Figure 2). All of these impurities have been detected in varying levels in clandestinely prepared (+)-methamphetamine. When synthesizing methamphetamine using this approach or amphetamine from phenylpropanolamine, retention of configuration at the amine carbon occurs. The enantiomeric composition of illicit amphetamine or methamphetamine is rarely reported.

Additional amphetamine analogs identified in the illicit drug market since January 1984 are N,N-dimethylamphetamine (synthesized from N-methylephedrine using HI and red phosphorous) (Tackett et al. 1988, Bond 1988), N,N-dimethylphenethylamine (Clark 1986), N-ethylamphetamine (DEA Dec. 1985), 4-methyoxyamphetamine (DEA Aug. 1985), 4-methoxymethamphetamine (DEA July 1987), N-2-hydroxyethylamphetamine (Heagy and Allen 1987). N-2-cyanoethylamphetamine (DEA Aug. 1987), and 4,5-dihydro-4-methyl-5-phenyl-2-oxazoline (U4Euh, prepared by the condensation of phenylpropanolamine with cyanogen bromide) (Inaba and Brewer 1987, Davis and Brewster 1988). The impurities from manufacturing have not been reported for these compounds.

The pharmacology reported for the synthetic contaminants in amphetamines is very limited except for 2-(phenylmethyl)phenethylamine (Soine 1986). In a recent study the intraperitoneal LD_{50} and CD_{50} (convulsive dose 50) of 2-(phenylmethyl)phenethylamine and N-methyl-2-(phenylmethyl)phenethylamine in mice was evaluated in relation to amphetamine and methamphetamine (Noggle et al. 1985). The LD_{50} values for both contaminants were comparable to that of both amphetamine and methamphetamine, however, the conta-

minants exhibited different non-lethal symptoms compared with animals given the corresponding amphetamines. In doses well below the LD_{50} the contaminants exhibited marked convulsive episodes characterized by clonic and tonic seizures which suggest greater CNS stimulation by the contaminants at the brain stem and cord levels. These symptoms were seen for the amphetamines only at doses at or near the amphetamine LD_{50} doses. The information concerning the pharmacology of the other synthetic impurities is minimal (Soine 1986).

COCAINE

Cocaine is obtained from the leaves of coca plants belonging to the genus Erythroxylum (family Erythroxylacae) with the major species used for the legal and illegal production of cocaine being E. coca Lam. var. coca ("Bolivian" or "Huanuco coca") (Novak et al., 1984). In general the primary method for preparing illicit cocaine in the coca-producing area involves crushing and soaking the leaves in a Na_2CO_3/kerosene mixture. Upon filtration, the kerosene filtrate is extracted with dilute H_2SO_4. The acid extract is made basic and the solid precipitate obtained is referred to as coca paste or cocaine sulfate base. Coca paste varies in cocaine content from 30-90% cocaine. This coca paste is further refined in cocaine "crystal" laboratories. During this process the coca paste is dissolved in a dilute H_2SO_4 solution, the insolubles are filtered off and the acid solution is made basic with ammonium hydroxide or an ammonium hydroxide/$NaHCO_3$ buffer. The cocaine base, which is relatively pure (90-95%), precipitates. The cocaine base is dissolved in acetone/ether to which concentrated HCl is added from which very pure cocaine hydrochloride precipitates. Depending on the skill of the operator and the stage at which the sample is obtained, numerous other alkaloids, in addition to cocaine, will be present. The alkaloids containing a tropane nucleus include benzoylecgonine, methylecgonine, ecgonine, cis- and trans-cinnamoyl cocaine, α- and ß-truxillines, and tropacaine. Due to crystallization behavior similar to cocaine, the cinnamoylcocaines are frequently detected, and are usually present in very low concentrations (less than 5%) (Soine 1986).

Due to the crude processing facilities that are used, residual solvents are usually present in illicit cocaine. Because these solvents may be indicative of solvent requirement or if samples are being obtained from a common source the DEA has carried out studies characterizing the volatile components present in cocaine HCl. Prior to 1981 all cocaine samples contained trace levels of acetone and ether. Recent studies indicate that acetone/ether is still preferred for cocaine processing (49% of the samples). However, a decreasing availability of diethyl ether in South America appears to have forced the usage of other solvents such as acetone , methylethylketone (Churchill 1985) and benzene (Kiser 1986;. Volatile compounds that are also associated with illicit cocaine samples are the hydrocarbons present in kerosene (alkylbenzenes and hydrocarbons), contaminants of acetone (mesityl oxide and diacetone alcohol), and trans esterification

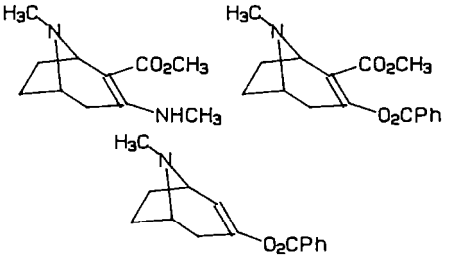

Figure 3
products from cocaine (methyl benzoate, methyl cinnamate and dimethyltruxillate) (Pettit 1986).

Numerous methods for the total synthesis of cocaine have been reported in the scientific literature and many of these methods have been attempted by the clandestine chemists (Cooper and Allen 1984). When cocaine is obtained by total synthesis it is referred to as "synthetic" cocaine. To date all seizures of operating clandestine laboratories have utilized routes through the common intermediate, 2-carbomethoxytropinone. The most commonly observed route is that described in "head shop" publications which contain reprints of the work by Preobazhenski (1936). The least commonly encountered method, although it is usually used by the most experienced chemists, is the method described by Findlay (1957). "Synthetic" cocaine is identified by the presence of the diastereomers of cocaine (pseudococaine, allococaine or allopseudococaine) or by the d enantiomer of cocaine. Additional contaminants common to most of the synthetic routes are shown in Figure 3. Contaminants that are present in both synthetic and natural cocaines are benzoyl pseudotropine and benzoyl tropine.

The biological activity associated with the naturally occurring alkaloids of cocaine (other than cocaine and nicotine) have been comprehensively reviewed up to 1984 (Novak et al., 1984). Based on this review no recent reports are available concerning the pharmacological activity of the tropane alkaloids methylecgonidine, pseudotropine, α- and ß-truxilline, and the pyrollidine alkaloids hygrine, hygroline and dihydrocuscohygrine. Cinnamoylcocaine had previously been reported to have no pharmacological activity (Woker 1953). Recent studies have shown that cinnamoylcocaine(s) suppress the primary humoral (PFC) immune response of mice following oral administration (Watson et al., 1983). The presence of a potential Michael adduct in cinnamoylcocaine could be associated with this response, however, this is unlikely since this effect was less than that observed for cocaine at the same dose. In in vitro studies (-)-trans-cinnamoylcocaine was one order of magnitude less potent than cocaine in binding to the

central high-affinity [^{3}H]-cocaine binding site, but was comparable to cocaine in binding to the peripheral (liver) high-affinity cocaine binding site (Calligaro and Eldefrawi 1987). The α- and ß-truxillines are reported to have no anesthetic action although they were originally reported as strong heart toxins (Liebermann 1888). The activity associated with the remaining ecgonine alkaloids suggest that these compounds do not contribute significantly to the CNS effects associated with cocaine (Novak et al. 1984).

It is hoped that this review will bring attention to some variables that can influence the pharmacological effects of clandestinely prepared drugs. Illicit drugs are usually impure and will predictably contain certain synthetic contaminants. Unfortunately, very little is known concerning the acute and chronic effects of these compounds.

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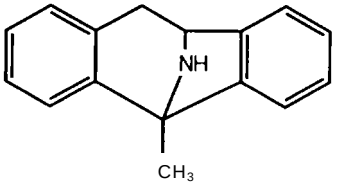
Affiliation: Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23298

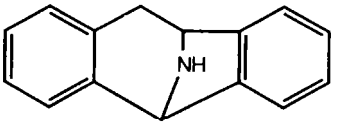
Structural and Conformational Aspects of the Binding of Aryl-Alkyl Amines to the Phencyclidine Binding Site

Andrew Thurkauf, James Monn, Marienna V. Mattson, Arthur E. Jacobson and Kenner C. Rice

Relatively recently, the dibenzo[a,d]cyclohepten-5,10-imines have been introduced into the family of structures which have appreciable affinity for the phencyclidine binding site of the mammalian CNS with the discovery that the anticonvulsant MK-801 (Dizocilpine)[1] showed high affinity for the site (K_i = 3 nM). This class is somewhat unique from other classes of PCP agonists in that it demonstrates the highest PCP affinity of the various structure, while having structural simplicity and the least conformational mobility.



MK-801 (Dizocilpine)



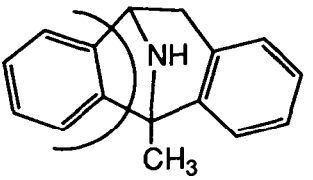
des-5-methyl MK-801

Structurally, the compound is an amino bridged dibenzocycloheptane. Previous work in our laboratories has shown that the structure may be simplified further, by removal of the C-5 methyl group giving des-5-methyl MK-801, and still maintain an affinity for the binding site (K_i - 120 nM) comparable to phencyclidine.[2]

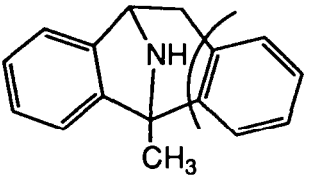
In an attempt to elucidate the structural requirements for binding in this series, we undertook a study of partial structures of des-methyl MK-801. Although structurally simple, the polycyclic nature of MK-801 permits it to be viewed as a derivative of various partial structures. In analyzing the molecule we choose that set of substructures which included both phenyl and the amino group.

As a bicyclic structure, MK-801 may be viewed as a tetrahydroisoquinoline or as a dihydroisoindoline. The acyclic substructures which can be visualized are the 1,2diphenylbenzylamine or a-phenylbenzylamine.

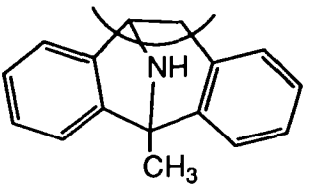
Substructures of MK-801



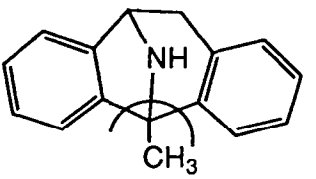
tetrahydroisoquinoline



dihydroisoindoline



a-phenylbenzylamine



1,2-diphenylethylamine

The acyclic structures examined are shown in Table 1. While α-phenylbenzylamine **(1)** shows little affinity for the PCP binding site, 1,2-diphenylbenzylamine **(2)** retains considerable affinity for the site. The partial structure, **(3)** and the homologue **(4).** also show little affinity for the site. The importance of the 2-phenyl ring to the binding of 2 is demonstrated by the poor affinity of the partially saturated analogue **(5).**

The bicyclic derivatives examined are shown in Table 2. While tetrahydroisoquinoline **(6)** itself shows no affinity for the PCP site, the 1-phenyl **(7)** and lbenzyl **(2)** derivatives do interact to some extent. Note that compound **(8),** which can be viewed as a conformationally restricted 1,2-diphenylethylamine, has somewhat greater receptor affinity than **(7),** a conformationally restricted a-phenylbenzylamine.

Table 1. Acyclic Analogues

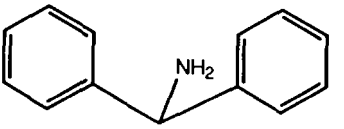
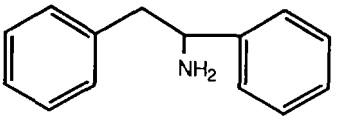
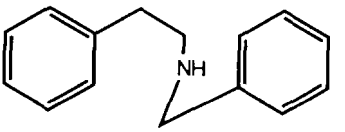
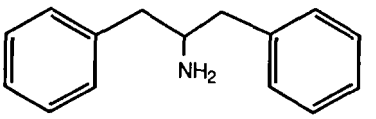
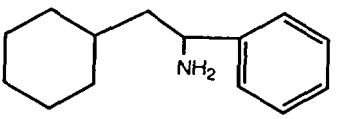
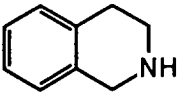
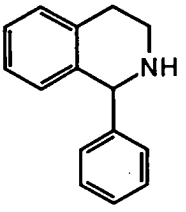
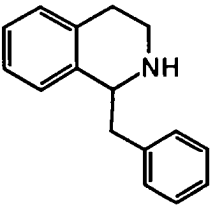
Comp. #		K_i (μM)
1.		20
2.		0.69
3.		100
4.		45
5.		33

Table 2. Bicyclic Derivatives

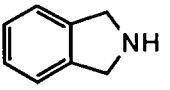
Tetrahrdroisoquinoline Derivatives

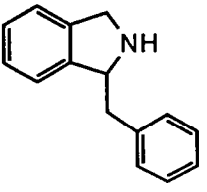
6.  K_i = >100 µM

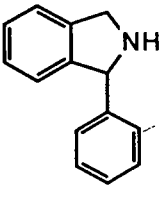
7.  K_i = 2.1 µM

8.  K_i = 1.3 µM

Dihydroisoindoline Derivatives

9.  K_i = >100µM

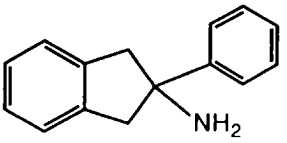
10.  K_i = 2.0 µM

11.  IC_{50} = >30 µM

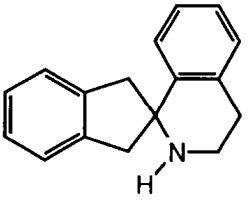
In the dihydroisoindole series, the parent compound (9) and the 1-phenyl derivative **(11)**[3] both show no affinity for the PCP binding site while the 1-benzyl derivative **(10)** retains some affinity for the site. As was observed in the tetrahydroisoquinoline series, the conformationally restricted; 1,2-diphenylethylamine derivative **(10)** is more potent, in vitro, than the conformationally restricted a-phenylbenzylamine **(11).**

The greater relative potency of derivatives possessing the 1,2-diphenylethylamine substructure over those of the three alternative substructures prompted us to further examine this system. Three conformationally restricted analogs of 1,2-diphenylethyl amine which were examined are displayed

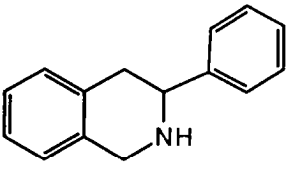
Table 3. Conformationally Restricted 1,2-Diphenylethylamines

12. 

K_i = 50 µM

13. 

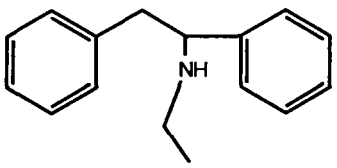
K_i = 20 µM

14. 

IC_{50} = 3.9 µM

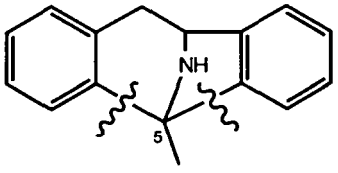
in Table 3. In compounds **(12)** and **(14)**[3], the rotation of 2-phenyl ring is restricted, while in compound **(13),** the rotation of both phenyl rings is restricted. While the affinities of all of these compounds are reduced, relative to the parent compound 1,2-diphenylethylamine **(2),** compound **(14)** with a secondary nitrogen (as in MK-801). has greater affinity than those restricted at the carbon adjacent to the nitrogen.

With the idea that secondary amines were not inimical to the effect of the 1,2-diphenylamine derivatives, we prepared the acyclic compound **(15).** Structurally, **(15)** can be visualized as MK-801 with the C4a-C5 and C5-C6a bonds removed. This compound showed very respectable affinity for the PCP binding site.



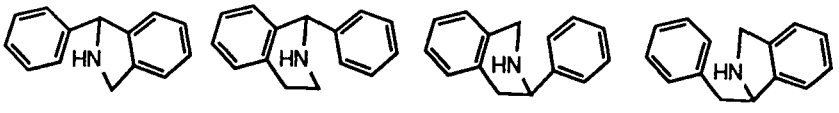
15.

K_i = 257 nM



MK-801

In conclusion, we have synthesized a number of partial structures of the phencyclidine agonist des-5-methyl MK-801. The structural and conformational requirements for binding are strict within this series. Analogues in which one of the phenyl groups is allowed free rotation showed greatly diminished receptor affinities.



Finally, the structural studies indicate that, for the purposes of medicinal chemistry, MK-801 is best viewed as a conformationally restricted 1,2-diphenylethylamine derivative.

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Acknowledgements

The authors would like to thank Dr. Patrick Mariano of the University of Maryland for a sample of Compound 13.

Affiliation

Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Desipramine Treatment for Relapse Prevention in Cocaine Dependence

Susan L. McElroy, Roger D. Weiss, Jack H. Mendelson, Siew K. Teoh, Brenda McAfee and Nancy K. Mello

Six open (Brotman et al., 1988, Gawin and Kleber 1984, Giannini et al., 1986, Kosten et al., 1987, Rosecan 1983, Tennant and Rawson 1983) and 5 controlled (Arndt et al., 1989, Gawin et al., 1989, Giannini and Billett 1987, O'Brien et al., 1988, Tennant 1984) studies have assessed the efficacy of tricyclic antidepressants for the treatment of cocaine dependence. Nine of these trials (Brotman et al., 1988, Gawin and Kleber 1984, Gawin et al., 1989, Giannini and Billett 1987, Giannini et al., 1986, Kosten et al., 1987, O'Brien et al., 1988, Rosecan 1983, Tennant and Rawson 1983) have shown that tricyclics may facilitate abstinence in cocaine-dependent patients by reducing dysphoria (Brotman et al., 1988, Giannini and Billett 1987, Giannini et al., 1986, O'Brien et al., 1988) and/or cocaine craving (Brotman et al., 1988. Gawin and Kleber 1984, Gawin et al., 1989. Kosten et al., 1987) associated with cocaine abuse (Gawin and Kleber 1986). Although the dosages and time course of treatment response reported in these studies paralleled the antidepressant properties of these drugs, the anti-craving and mood-elevating effects were observed in cocaine abusers independent of a concurrent diagnosis of major mood disorder (Gawin et al., 1989, Rosecan 1983). Moreover, in 1 of the 2 negative studies (Arndt et al., 1989, Tennant 1984) (double-blind, placebo-controlled trials with desipramine), both the medication dose and the duration of treatment were probably subtherapeutic (Tennant 1984).

In all of the reported studies, antidepressants were administered either during or shortly after a period of active cocaine use. To our knowledge, there has been only 1 report discussing the potential usefulness of antidepressants for the prevention of relapse to cocaine abuse after abstinence had been achieved. Weiss recently described 3 cocaine abusers who had been abstinent for 1 to 6 months and relapsed to cocaine use soon after initiating treatment with desipramine (Weiss 1988). Development of an "early tricyclic jitteriness syndrome" (Pohl et al., 1988. Pollack and Rosenbaum 1987) and increased craving for cocaine were believed to be the likely precipitants of relapse in these cases. In order to systematically investigate the efficacy of desipramine for preventing relapse in cocaine abusers, we performed a double-blind, placebo controlled 6-month crossover pilot study of desipramine in 15 cocaine dependent patients who had attained 4 to 8 weeks of abstinence by completing an inpatient substance abuse treatment program.

METHODS

All study patients were hospitalized for cocaine dependence at the Alcohol and Drug Abuse Treatment-Center of McLean Hospital, Belmont, Massachusetts, where they participated in a multidisciplinary substance abuse treatment program. The average length of stay in the program was 4 weeks, although some patients stayed for up to 8 weeks. Abstinence during the hospitalization was monitored by random urine drug screening. Patients who had positive urine tests indicative of in-hospital drug use were ineligible for the study. From September, 1986 through September, 1988, patients who met the following criteria were recruited to participate in the study: a diagnosis of cocaine dependence meeting DSM-III-R criteria; age between 18 and 65 years; no significant medical or neurological problems; no history of major mood, anxiety, eating, or psychotic disorders; consent for urine screens and blood tests; and no past therapeutic trials of thymoleptic medication. Patients were included in the study if they met DSM-III-R criteria for other psychoactive substance use disorders, as long as cocaine was their preferred and most prominent drug of abuse. Patients were also included if they had a past or current diagnosis of attention deficit disorder with hyperactivity.

After providing informed consent, patients were randomly assigned to receive desipramine or placebo. Medication was begun approximately 1 week prior to discharge, after completion of the 4 to 8 week period of abstinence and inpatient treatment. Patients were administered identical-appearing capsules of desipramine or placebo. Desipramine was started at 50mg a day and increased by 50mg each day to a daily dose of 200mg. Patients were randomly assigned to receive desipramine or placebo during the first 12 weeks of the study. After completing the first 12 weeks, patients were then crossed-over to the other condition for the remaining 12 weeks.

Patients were assessed at baseline and weekly thereafter for 24 consecutive weeks. During weekly interviews any potential drug use during the past week was discussed and psychiatric symptoms and medication side effects were assessed. To measure cocaine craving, 100 mm line analog scale ratings for. intensity and frequency of cocaine craving were completed. Patients were instructed to mark the intensity and frequency of their craving for the past week on a 100 mm line, with 0 representing the absence of craving and 100 representing "more craving than ever." The Beck Depression Inventory (BDI) (Beck et al., 1961). the Hamilton Depression Rating Scale (Ham-D) (Hamilton 1960). a urine toxicology screen, and a side effects scale were also completed. Relapse to cocaine use was ascertained by both clinical interviews and urine toxicology screens. Routine blood tests (CBC, SMA-12) were obtained every 2 weeks. If patients relapsed to cocaine abuse, they were still encouraged to remain in the study.

RESULTS

Demographic and clinical characteristics of the 15 patients are presented in table 1. Eleven patients were men and 4 were women. The mean age of the patients was 29.5 ±: 5.7 years (range, 23 to 43 years). The average amount of cocaine used was 23.1 ± 31.5 grams/week (range, 2-125 grams/week); the average duration of cocaine use was 4.9 ± 3.6 years (range, 1 to 15 years). All patients used cocaine intranasally; 5 also smoked the drug and 4 also used it intravenously. All patients met DSM-III-R criteria for current or past abuse of

other drugs in addition to cocaine, and most patients participated in 1 or more modes of psychosocial treatment while enrolled in the study.

TABLE 1 Subject Characteristics[a]

Variable	Desipramine first (n=9)	Placebo first (n=6)	Differences
Age (years)	31.9 ± 6.1 (25-43)	26 ± 2.7 (23-31)	NS
Sex (% M/F)	78/22	67/33	NS
Amount of cocaine used prior to hospitilization (grams/week)	29.3 ± 37.8 (4-25)	13.8 ± 17.71 (2-49)	NS
Duration of cocaine abuse (Years)	5.6 ± 4.3 (1.5-15)	3.7 ± 2.3 (1-6)	NS
Baseline cocaine craving-intensity[b]	34.2 ± 34.1	31.5 ± 17.7	NS
Baseline cocaine craving-frequency[b]	21.9 ± 24.1	15.8 ± 11.3	NS
Baseline Hamilton Rating Scale for Depression score	6.6 ± 4.8 (1-16)	7.8 ± 5.9 (2-17)	NS
Baseline Beck Depression Inventory score	4.0 ± 4.8 (0.5)	9.5 ± 10.1 (1-24)	$p < .05$
Duration of time in study (weeks)	11.0 ± 10.7 (1-24)	6.5 ± 5.4 (1-14)	NS

[a] Values not identified as percentages are express as mean ± SD (range).

[b] The range of the cocaine craving measure for both intensity and frequency is 0 to 100, with 0 representing "no craving" and 100 "more craving than ever."

Of the 15 patients, 9 were randomized to receive desipramine and 6 placebo during the first 12 week phase. As shown in table 1, there were no significant differences between those patients initially randomized to drug and those to placebo with respect to sex, age, amount of cocaine used, duration of use, route of use, concurrent or past alcohol or substance abuse, baseline measures of cocaine craving, intensity and frequency. or baseline Ham-D scores. However, the 2 groups differed significantly with respect to baseline BDI scores, with the despiramine-first group being less depressed ($p < .05$) than the placebo-first group.

Only 3 (20%) patients completed the entire 24 weeks of treatment, The remaining 12 (80%) discontinued the study prematurely. Two of the latter patients had crossed over from one treatment to the other. One patient dropped out after 15 weeks of treatment, 3 weeks after being switched from desipramine to placebo, and could not be contacted to determine his reasons for dropout. A second patient terminated after 14 weeks, 2 weeks after being switched from placebo to active drug, citing dry mouth, bad taste, feeling overly stimulated and jittery, and experiencing increased craving for cocaine as the reasons for drop out. The remaining 10 patients left the study prematurely after completing 12 weeks or less of treatment; 6 patients terminated after completing 2 weeks or less. In total, 6 of the 12 non-completers (50%) dropped out while on placebo and 6 (50%) dropped out while on active drug. Reasons for failure to completed the entire 24 weeks of the study included relapse to cocaine abuse in 5 cases (3 on placebo and 2 on drug); uncomfortable or intolerable side effects without relapse in 3 cases (all of desipramine); emergence of major depression without

relapse in 1 case (on placebo); no longer wanting to participate in the study because of time constraints in 2 cases (1 on placebo and 1 on drug, no relapses); and failure to make appointments and loss of contact in 1 case (on placebo). AU patients who relapsed to cocaine use discontinued the study.

Relapse to Cocaine Use

While they were participating in the study, 2 of 9 patients (22%) relapsed while on desipramine and 3 of 6 (50%) relapsed while on placebo. This difference failed to achieve statistical significance. However, this may be due in part to the small sample size.. Relapse was not significantly correlated with age, sex, amount or duration of cocaine abuse, baseline measures of cocaine craving and mood, or duration of time in the study. Given the extremely high drop out rate, however, it was difficult to accurately assess relapse over the long term. All 5 patients who, to our knowledge, relapsed to cocaine use did so while participating in the study and within 4 weeks following hospital discharge. We attempted to interview the other 10 patients by telephone to determine whether or not they had relapsed to cocaine use after discontinuing the study. Of 8 patients contacted at follow-up periods of 4 to 28 months after study termination, all denied relapse to cocaine use.

Cocaine Craving and Mood

An analysis of variance for repeated measures was carried out to determine if there were any significant changes in cocaine craving intensity (CCI), cocaine craving frequency (CCF), BDI scores, and Ham-D scores over time. However, this was not possible for the entire patient group because of insufficient data due to the high drop out rate. The analysis was then repeated for patients who completed the first 6 weeks of the study. There were no significant changes in CCI, CCF, BDI scores, and Ham-D scores for the 3 patients who received placebo for the first 6 weeks of the study. Similarly, there were no significant changes in CCI, CCF, and BDI scores for the 5 patients who received desipramine for the first 6 weeks of the study. However, there was a significant decrease in Ham-D scores at weeks 3 and 4 when compared to baseline scores ($p < .05$) for these patients.

Premature Study Termination by Patients Because of Adverse Effects

Premature termination due to adverse effects occurred more frequently during treatment with desipramine than with placebo. However, this difference did not achieve statistical significance. Termination secondary to adverse effects was not significantly correlated with age, sex, amount or duration of cocaine abuse, baseline measures of cocaine craving and mood, or duration of time in the study. All 3 patients who dropped out because of adverse effects were receiving desipramine at the time and reported either feeling overly stimulated, jittery and/or anxious, feeling “high,” a “rush,” or a “crash” as if they were using or had just used cocaine, and/or increased cocaine craving (all 3 patients) as the primary reasons for ending the study. These side effects began within 1 to several days after beginning desipramine therapy. Only 1 of the other 7 patients ever receiving desipramine reported feeling stimulated and anxious. This was not accompanied by feelings of cocaine intoxication and subsided after 2 weeks. The 2 patients who relapsed to cocaine use while on desipramine did not report adverse effects.

DISCUSSION

Although more patients relapsed to cocaine use when treatment was begun with placebo than with desipramine. this finding did not achieve statistical significance. Our data suggest that treatment of cocaine-dependent patients with desipramine - initiated well after induction of abstinence - may not be helpful in prevention of relapse. This conclusion is inconsistent with many studies that indicate that antidepressants may be helpful in facilitating abstinence in active cocaine abusers (Brotman et al., 1988, Gawin and Kleber 1984, Gawin et al., 1989, Giannini and Billett 1987, Giannini et al., 1986, Kosten et al., 1987, O'Brien et al., 1988, Rosecan 1983, Tennant and Rawson 1983). A number of these studies (Brotman et al., 1988, Gawin and Kleber 1984, Gawin et al., 1989. Giannini and Billett 1987. Giannini et al., 1986, Kosten et al., 1987, O'Brien et al., 1988) have shown that the reduction in cocaine use during antidepressant treatment is associated with reductions in cocaine craving or post-use dysphoria. The effectiveness of pharmacologic treatment in these studies may thus have been due to alleviation of cocaine withdrawal symptoms. However, in the present study, patients who completed the first 6 weeks of treatment did not report significant changes in craving for cocaine. Hamilton depression (Ham-D) scores but not Beck depression (BDI) scores were significantly decreased at weeks 3 and 4 in comparison to baseline. There were no significant changes in Ham-D and BDI scores for the 3 patients who received placebo during the first 6 weeks of the study, even though baseline BDI scores were significantly higher in the placebo-first group. These findings suggest that although treatment with desipramine reduced depression, it did not promote relapse prevention perhaps because cocaine craving was not reduced. Thus, desipramine treatment may be more effective in facilitating rather than maintaining abstinence from cocaine dependence because cocaine withdrawal symptoms (particularly craving for cocaine) may diminish during protracted abstinence (Gawin and Kleber 1986), when other factors may be more important in precipitating relapse.

Our findings and conclusions, however, are limited by the design of the study. First, despite random assignment, the desipramine-first and placebo-first groups were not identical. Although the 2 groups of patients were similar with respect to CCI, CCF, and Ham-D scores, the drug-first group had significantly lower BDI scores. Since a recent report by Carroll (1989) has suggested that concurrent depression may be a good prognostic sign in cocaine abusers, patients who were given desipramine initially may have been more likely to relapse because of a reduced degree of depression. This possibility seems unlikely, however, since relapse was not correlated with depression scores in our study.

Desipramine may have facilitated maintenance of abstinence for some patients (4/9 or 44%), but it did not prevent relapse for other patients, (2/9 or 22%). The drug also caused adverse effects (similar to the early tricyclic "jitteriness" syndrome) in 4 of 9 (44%) patients. While these adverse effects did not invariably lead to relapse, in contrast to the report by Weiss (1988), 3 of 4 (75%) patients discontinued the drug, fearing that persistence of these adverse effects would cause resumption of cocaine use. Thus, desipramine may be helpful for some cocaine abusers, but it also may be deleterious for others - especially at the initiation of pharmacotherapy, when it may induce effects similar to cocaine intoxication.

Since neither relapse nor discontinuation of medication due to adverse effects was associated with specific clinical or demographic factors (e.g., age, sex, severity of cocaine dependence, severity or frequency of cocaine craving, severity of depression), we are unable to identify predictors of favorable or negative desipramine effects in cocaine abusers. Since relapse was less common (although not significantly) when treatment was initiated with desipramine than with placebo, desipramine treatment may be more likely to prevent relapse when administered during the early rather than later phase of cocaine abstinence.

In summary, this study suggests that treatment of abstinent cocaine-dependent patients with desipramine may not be effective in preventing relapse to cocaine use or reducing craving for cocaine - even though it may reduce depression. This finding must be considered tentative, however, in light of the study's design problems - unequal groups, high attrition rate, and small sample size. Nonetheless, in some patients, treatment with desipramine appeared deleterious, with induction of symptoms suggestive of the early tricyclic "jitteriness" syndrome and increased craving for cocaine. When deciding whether or not to treat cocaine dependence with desipramine, it will be important to realize that some patients - particularly those who have achieved abstinence through psychosocial means - may sometimes experience symptoms increasing the risk of relapse. Currently, it is impossible to predict which patients with cocaine dependence will respond to desipramine favorably or deleteriously. Further research on the efficacy of tricyclic antidepressants in relapse prevention in cocaine dependence is needed to identify those subgroups of cocaine abusers who respond favorably to this class of medications.

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ACKNOWLEDGEMENTS
This study was supported by grants DA04059, DA00064 and DA00101 from the National Institute on Drug Abuse.

AUTHORS
Susan L. McElroy, M.D., Roger D. Weiss, M.D., Jack H. Mendelson, M.D., Siew K. Teoh, M.D., Brenda McAfee, Nancy K. Mello, Ph.D.
AFFILIATION
Alcohol and Drug Abuse Research Center, McLean Hospital/ Harvard Medical School, 115 Mill Street, Belmont, MA 02178

NIDA's Medication Development Program - 1989

Charles R. Schuster and Marvin Snyder

The development of improved treatment for drug addiction is one of the most difficult problems confronting medical science today. With the staggering cost of drug abuse and the burdensome future liabilities associated with the spread of human immunodeficiency virus (HIV) infection within the drug abusing population, effective drug addiction treatment is necessary now, more than ever. When NIDA alerted the Congress to the possibility of new pharmacotherapeutic approaches to drug addiction treatment, the Congress responded by specifying the need for the development of new medications in the Anti-Drug Abuse Act of 1988. NIDA has consequently made medications development one of the top research and programmatic priorities of the Institute, in line with the Public Health Service Plan for Reducing the Demand for Illicit Drugs.

Drug abuse is a unique problem; individuals seek to alter, or indeed to "experiment" with the functioning of the nervous system in an attempt to change how they feel and how they see the world and react to it. There is a distinction, however, between drug abuse and drug addiction. Drug abuse is a voluntary behavior; the casual user makes a free and conscious decision to break the law and use mind-altering substances. Drug addiction is a disease of the brain, resulting from repeated self-administration of these substances.

Our brains are made up of billions of neurons. These cells activate one another through the release of chemicals, and this brain activity enables us to function: to see, to hear, to feel anger, and to experience hunger for food, water, and sex. Abused drugs disrupt brain function, short circuit, if you will, the brain's normal activity, so that the individual becomes pathologically preoccupied with repeating the drug experience, either for the pleasure it brings or the pain it relieves. Such individuals have diminished freedom of choice. They are not irrational but they are dysfunctional. Their waking hours are focused on obtaining, selling, thinking about, and experiencing drugs. One of the roles of medications in these individuals is to restore a degree of normality to brain

function and behavior so that they have the opportunity for rehabilitation through counseling, psychotherapy, vocational training, and other therapeutic services.

We pointedly use the term "medication" for that is what we propose to develop. We have been unduly burdened by the fact that we do not have different words to refer to chemicals used therapeutically as opposed to those being abused. Other languages, such as French, make a clearer distinction. Unfortunately, semantics sometimes gets in the way of reality, and there are those who say it is "silly" to talk about using "drugs" to treat drug abuse. In reality, nothing could be further from the truth. Drugs or medications are typically used to treat the biochemical and physiological imbalances caused by a variety of diseases. Treatment of psychiatric conditions, like anxiety, depression, and schizophrenia is perhaps most relevant here. Correcting the biochemical imbalances in the brain that lead to the disordered behaviors seen in these conditions not only improves the patient's emotional state, but also improves behavior and increases the effectiveness of other forms of treatment, like psychotherapy. As just mentioned, we will be developing therapies to restore some normality to brain function disrupted by the abuse of illicit drugs. We will be using the word medication to help keep the approach and the distinction between abused drugs and medications clear.

Drug abuse research has led to fundamental insights about drug-receptor interactions, the structure-activity relationships of drugs and the brain, and mechanisms underlying neurotoxicity. With this information and utilizing state-of-the-science technological advances in areas like molecular biology, neural imaging techniques, and computer-aided drug design and modelling, we plan to develop medications that:

1. reduce the craving for abused drugs;

2. block the behavioral and physiological effects of abused drugs;

3. safely serve as replacement therapies for the treatment of drug addiction,

4. moderate and/or climinate the process of withdrawal;

5. block and/or reverse the toxic effects of abused drugs; and

6. prevent, under certain conditions, the initiation of drug abuse in individuals at high risk.

The development of pharmacotherapies for the treatment of drug addiction has not received significant support from the pharmaceutical industry. There are many reasons for this

unfortunate circumstance. First, drug abusers are a unique group of sick people who do not necessarily seek treatment and, indeed, may actively avoid it. Research on this population is difficult and is not attractive to many investigators. Second, prior to the current cocaine epidemic, the number of drug abusers, although large, was not of sufficient size to be economically rewarding to pharmaceutical manufacturers and the profit potential of new treatments was not great. Furthermore, pharmaceutical manufacturers have noted that both the distribution and reimbursement for new medications are likely to be carefully controlled, further reducing their attractiveness. Third, some drugs that could be developed are either off patent or about to come off patent, and, therefore, there is limited protection for the product and, hence, little profit potential. Fourth, drug companies are concerned about having their product lines associated with drug abusers ("junkies") in the public's mind. Fifth, drug companies are concerned that by introducing a product into the drug abuse population they might decrease the market for the product's other potential uses or delay its approval. The product, for example, may be placed in an unacceptable schedule under the Controlled Substances Act or its use restricted, like methadone. Finally, pharmaceutical manufacturers are concerned about a large variety of liability issues, some of which may be unique to drug abusers.

Although some of these concerns may be overrated, others are real. The risk involved in developing treatment medications for pregnant drug abusers, for example, has to be considered excessive by even the most compassionate and concerned corporate officers. From a public health perspective, however, we believe that society has been ill-served by the lack of attention paid by the industry to the problems of drug addiction. The existence of only two approved medications, methadone and naltrexone, available specifically for drug addiction, is a testament to this failure.

However, the incentives of the Orphan Product Act, AIDS, and the size and demographics of the cocaine epidemic are changing this situation. Some discussion has taken place as to whether the Act might be extended to all medications being developed for the treatment of addictive disorders. Even though some of the addictive disorders do not meet the technical criteria for orphan conditions, the lack of private sector effort in developing medications to treat the addictions suggests that they are, in effect, orphan conditions. NIDA staff have been visiting and discussing medications development with a number of pharmaceutical firms, and we believe that there is increasing interest in collaborative efforts and that these collaborations will grow with time. It is nonetheless clear that significant leadership is required, in this area - leadership that NIDA plans to provide by committing itself, on a long-term financial, programmatic, and scientific basis, to the development of medications for the treatment of drug abuse.

Starting in fiscal year 1989, NIDA expanded its efforts in the area of medications development by establishing a research program specifically seeking to identify new medications for drug addiction treatment. We are quite mindful of the fact that many of the chemicals that will be identified and/or synthesized will, through their central nervous system (CNS) actions, have potential value for other disease conditions affecting the brain and behavior. We are, therefore, working closely with our Agency leadership and sister Institutes, the National Institute on Alcohol Abuse and Alcoholism and the National Institute of Mental Health, so that the opportunities to test new medications for both addictive and mental disorders will not be lost.

Compounds for evaluation will be derived from a comprehensive database that NIDA is developing about chemical entities with CNS activity. The information in the database will include pre-existing compounds, as well as compounds developed on the basis of computer-assisted drug design and knowledge of structure-function activity, receptor pharmacology, and the functional mechanisms underlying drug reinforcement, euphoria, dysphoria, tolerance and dependence.

New potential medications that meet our basic pharmacological parameters for candidate treatment agents will then be evaluated for potential efficacy and safety in animal models through ongoing contractual relationships. The animal self-administration model for drug abuse is potentially one of the best models of human disease available. It is highly reliable in predicting the abuse liability of new compounds and it should prove an invaluable tool for our treatment development program. The evaluation of many proprietary compounds may be modeled after the abuse liability testing program that is currently used to make regulatory and scheduling decisions about new drugs. Although NIDA now funds this abuse liability testing program, we do not control it directly. NIDA will be more directly involved in the new program for testing potential treatment medications. The development of specific relationships with individual pharmaceutical companies that will allow the Federal Government to test and evaluate privately owned compounds and to use the data for medications development is now being explored.

Three issues surrounding the potential toxicity of new compounds are of particular concern. Because we will be dealing with substances that act on the CNS, and because recent findings show that a variety of centrally acting compounds are potentially neurotoxic, we will be paying particular attention to both the acute and long-term effects of candidate medications on the brain. Second, the medications we are developing are primarily being targeted to patients who are either seropositive for HIV or at high risk of exposure to HIV. We are particularly concerned that we do not administer anything to these people that might accelerate the clinical course of

the disease, increase the probability of clinical infection after exposure, or interfere with anti-viral therapy. Given the limited knowledge of the natural history of AIDS and the role of co-factors in the disease, it is not clear how we will do this. Nonetheless, it is an area of significant priority that we are examining.

Finally, we are facing another unusual situation in developing medications for the treatment of drug addiction. Drug addiction is a disease that involves the self-administration of a drug which, like cocaine, may produce an acute, fatal reaction. Because a number of candidate treatment agents might potentiate these reactions if taken with cocaine, we are working with the Food and Drug Administration (FDA) on the issue of requiring drug interaction studies to show safety of potential treatment agents.

In terms of clinical trials, in fiscal year 1989 NIDA will establish Treatment Research Units that will have a major role in evaluating new medications and conducting the necessary clinical trials for phases I and II of the drug development process. We also plan to establish in fiscal year 1990 agreements with a number of pharmaceutical firms that would agree to take promising new medications through phase III clinical trials, New Drug Application (NDA), and approval, and marketing of the drug in exchange for exclusive rights to the compound for the treatment of drug addiction. In other words, NIDA, as the lead Institute for the Agency, would, under some conditions, perform all necessary preclinical and phase I and phase 11 clinical studies and transfer these data to a pharmaceutical firm willing to complete the NDA process and market the drug. Approaches like this have already been used successfully at both the National Cancer Institute and the National Institute of Neurological Disease and Stroke, and we are hopeful that it can work for us.

We would now like to briefly describe some of our ongoing activities. Comprehensive discussion of the treatment of drug addiction must begin with methadone. Methadone is certainly not a cure for heroin addiction. It must be administered daily. It maintains physical dependence on opiates. It is subject to diversion and abuse. Children born to methadone-maintained women must be withdrawn from the drug. But methadone is a successful treatment for heroin addiction and a valuable tool for preventing the spread of AIDS. The evidence indicates that children born to methadone-maintained mothers are much healthier at birth than children born to heroin addicts. The concept of methadone maintenance is consistent with the medical management and treatment (not cure) of a number of other diseases, including diabetes, heart disease, and arthritis.

We believe that methadone maintenance has a place in the treatment of heroin addiction. The 25-year-old heroin addict

who comes into the clinic and is placed on methadone is no longer an addict. That person is physically dependent on opiates, but not addicted to them. This is an essential and not a semantic distinction. Methadone stabilizes brain dysfunction caused by years of heroin addiction and stops the on-off cycling that typifies addiction. This person no longer injects drugs, is no longer constantly involved in drug-seeking behavior, and can be gainfully employed and involved in rehabilitation programs. This person no longer needs to commit crime to support his or her drug addiction. Perhaps most importantly, the risk of contracting AIDS and/or transmitting it to someone else is substantially reduced.

Tragically, AIDS and drug abuse are now and will continue to be inseparable for the foreseeable future. Methadone maintenance reduces intravenous drug abuse and thus reduces the spread of AIDS. An unrealistic wait for a "cure" for heroin addiction and a philosophy that refuses to accept the medically sound concept of maintenance will needlessly sentence thousands. of people, including innocent children, to death.

Methadone was the first medication available for the treatment of heroin addiction. We need to continue its use until it is replaced by something better. Such progress is attainable through research. Medical science utilizes what it has and improves upon it through the development of new knowledge. The sulfa drugs were among the first true antibacterials. They were followed by antibiotics, such as penicillin and tetracycline, and then whole new classes of anti-infective agents, each one useful in specific patients, with specific diseases, and at specific times in their therapy. This is the goal of NIDA's Medications Development Program. We are attempting to develop new, more effective medications to treat addictive disorders, most notably heroin and cocaine addiction. We would like to be able to provide physicians with a wide variety of potential treatments so that these can be tailored to the needs of a particular patient.

With newly available funds, NIDA is renewing its sponsorship of LAAM for the treatment of heroin addiction. LAAM therapy, like methadone, is based on the concept of maintenance. We believe it will find greater public acceptance than methadone and that it will be a significant improvement over methadone for many patients. LAAM is a long-acting medication that needs to be taken only every 2 to 3 days. Many believe that this reduction in drug-taking behavior, by itself, will have therapeutic value because it will break the drug-taking cycle so characteristic of addiction. In addition, because each patient will visit the clinic less frequently, treatment capacity should increase and costs decrease. Most importantly, LAAM does not produce a high; it should have little, if any, abuse liability. Consequently, we do not anticipate any problems with diversion for street use.

We should know within a year if the available data show safety and efficacy. If so, NIDA plans to work with the FDA to make LAAM available as rapidly as possible and will explore the mechanisms of a treatment Investigational New Drug (IND) to accomplish this goal. This mechanism has been effectively used in the AIDS arena, and we believe it is appropriate to follow this approach with drug addiction. Under this program, patients would be treated with LAAM while it is still awaiting final approval by the FDA.

For those patients who wish to detoxify from heroin or be medically withdrawn from methadone, NIDA-sponsored research has been examining techniques and medications to facilitate this process. One such medication is an anti-hypertensive agent called clonidine which interestingly has shown value in the treatment of heroin addiction. Clonidine by itself and in combination with naltrexone appears to be quite helpful and is currently being used to assist patients to rapidly become free of physical dependence on heroin (Kleber et al., 1985; Charney et al., 1986).

For those patients who are opiate-free but require "protection" against re-addiction to heroin, NIDA developed, in a partnership with DuPont, the narcotic antagonist naltrexone. Patients whose heroin-free state is "maintained" by naltrexone Cannot get high from any street narcotic. Naltrexone seems, in many ways, to be an ideal medication. In effect, it "immunizes" against narcotic addiction. However, there have been significant problems with patient compliance. People don't want to, or forget to, take it. The patient has to be highly motivated to take a medication when its only action is to block the effects of heroin, a drug he's not supposed to be using. We believe however, that naltrexone offers substantial, unrealized benefits in our battle against both drug abuse and AIDS. We are, consequently, developing a "depot" form of this medication, which is slowly released over time. In this way, a single injection will provide protection against heroin for periods of 30 days or longer. The problems of patient compliance will be greatly reduced. If we can give patients a 30-day window of drug-free opportunity, there should be substantial improvements in treatment outcome. Clinical studies of this new formulation will begin late next year. Again, as with all of the medications under development, every effort will be made to make the naltrexone depot available as soon as possible in the development process.

We are also conducting clinical trials of buprenorphine, a new type of medication that combines some of the effects of opiate agonists with many of the effects of antagonists. This medication may be especially useful in helping addicts detoxify from heroin and may also serve as a useful maintenance agent. If ongoing studies at our Addiction Research Center prove successful, large-scale trials of buprenorphine should begin next year.

Buprenorphine may also be a useful treatment for combined heroin and cocaine addiction. In one study, patients who were being treated with buprenorphine for heroin addiction, and who were also cocaine addicts, were observed to reduce the abuse of cocaine (Kosten et al., 1989). In another study, recently published in the journal Science (Mello et al., 1989), monkeys that had been self-administering cocaine greatly reduced their use of it once they had been treated with buprenorphine. This is a very exciting, though still preliminary, finding. Needless to say, we will be following up on these discoveries as quickly as possible.

We would be remiss if we did not mention naloxone, a short-acting narcotic antagonist. This medication is used to treat the respiratory depression that follows a heroin overdose, and over the years has saved the lives of thousands of people. It is a success story that is frequently overlooked when we fail to see the addict as a person; someone with a life-threatening disease, who needs our help and our compassion.

What we have presented thus far is an abbreviated summary of our Medications Development Program's efforts to improve the treatment of heroin addiction. A significant amount of research is also ongoing to more precisely identify the mechanisms underlying drug addiction, and we are hopeful that this research will lead to innovative conceptual approaches to treatment.

An additional area of particular interest and concern involves the treatment of the pregnant addict. We are attempting to develop medications that will allow us to treat the mother without affecting the fetus. The scientific and technical problems are considerable but we think addressable. This is a new, high priority area that will involve a considerable amount of basic research.

Heroin addiction is very different from cocaine addiction. Although both substances are legally classed as narcotics, they represent two distinctly different kinds of abused substances. Heroin is a depressant drug. It acts directly on opiate receptors and thereby affects brain activity. Self-admimstration of heroin satisfies the need for heroin for periods of 4 to 6 hours. Cocaine is a stimulant drug. Its effects last for minutes rather than hours. In contrast to heroin, the self-administration of cocaine does not satisfy the need for cocaine. Rather, it stimulates craving for more cocaine. It acts indirectly on brain and behavior by modifying the release of other chemicals in the brain.

In light of what we understand about cocaine, the concept of maintenance therapy, vis-a-vis methadone, is not applicable to the treatment of cocaine addiction and is not consistent with our stated goal of normalizing brain function and behavior. We

are developing medications, however, that are designed to assist in treatment by a) blocking the euphoria and "rush" experienced by cocaine addicts, b)reducing the "crash" seen after stopping cocaine use, c) reducing the ensuing. depression and loss of the ability to experience pleasurable emotions, d) reducing the intense "craving" for cocaine, and e) acting as "antidotes" to toxic reactions and/or neurotoxicity.

There is new hope in regard to the treatment of cocaine addiction. We are beginning controlled clinical trials of two new medications that appear to hold considerable promise in treating intravenous cocaine and crack addiction. One medication, carbamazepine, has been studied at the National Institute of Mental Health for a number of years. Carbamazepine is approved as an anti-epileptic treatment, and it blocks the occurrence of seizures. Cocaine can produce these brain abnormalities and it has been suggested that a medication that controls these localized brain seizures might reduce craving for cocaine. One preliminary test of this idea proved successful in about one-third of the patients (Kuhn et al., 1989), and the studies are being enlarged as quickly as possible. One study will be run at NIDA's Addiction Research Center and another in one of our Treatment Research Units.

Another new medication, flupenthixol, showed even greater initial success in an open study of crack abusers (Gawin et al., 1989). This medication is proposed to normalize the disruptive effects of cocaine on dopaminergic systems. If flupenthixol proves to be safe and effective in our upcoming clinical trials, we will do whatever is necessary to ensure its rapid development and approval.

Let me emphasize that we do not envisage that our Medications Development Program will discover a magic bullet to cure drug addiction. We will develop medications that will help to normalize the brain and behavior of people made sick by drugs. As mentioned earlier, these medications will give us the opportunity to engage addicts in a variety of essential rehabilitative services. These services are crucial; they give the addict the skills needed to change destructive behaviors into adaptive behaviors. We will fail those addicts who seek help if we do not ensure that psychosocial counseling and rehabilitation services are available to all those patients we put on medications. Just as insulin will not save a diabetic who cannot change his diet and lifestyle, medications to to treat drug addiction. will not work by themselves. They must be part of a comprehensive treatment program that gives addicts the capabilities to achieve a life without drugs.

Medications play an essential role in our fight against disease. They are also vital part of the search for solutions to drug addiction. I hope that the scientists and physicians present at this meeting will join us in this initiative to

improve the treatment of drug addiction and prevent the spread of AIDS through effective pharmacotherapcutic interventions.

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AUTHORS:

Charles R. Schuster, Ph.D.
Marvin Snyder, Ph.D.
National Institute on Drug Abuse
5600 Fishers Lane
Parklawn Building
Rockville, Maryland 20857

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A Schema for Evaluating Methadone Maintenance Programs

John C. Ball

- The Status of Drug Abuse Treatment Evaluation

There is a particular need to evaluate the effectiveness of methadone maintenance programs in the United States in the AIDS era. This is because programs which reduce or eliminate IV drug use by patients can influence the spread of HIV in high risk metropolitan areas. Beyond the AIDS issue, there is a fundamental need to unravel the dynamics of treatment and rehabilitation in order to improve the effectiveness of drug abuse treatment generally.

A considerable body of scientific and medical knowledge has been published during the past twenty-five years about methadone maintenance treatment. Most of this research falls within two broad classifications: (1) pharmacological or clinical studies of the efficacy of specific drugs, dosages or related treatment regimens, (2) patient outcome studies which measure the overall effectiveness of methadone maintenance treatment. Investigators in both of these research areas have advanced our knowledge considerably and these contributions have been reviewed in a series of research monographs sponsored by the National Institute on Drug Abuse.

Nonetheless, in the past decade, researchers have noted that there is a pervasive lack of knowledge about how treatment is actually carried out in methadone maintenance programs. Indeed, this somewhat perplexing void in our knowledge of drug abuse treatment has been dubbed the "black box" of treatment.

- A Four Domain Schema for Treatment Evaluation

In order to conceptualize drug abuse treatment broadly and, thereby, to open the "black box" of treatment, an evaluation schema which includes four domains of data collection has been established. These four domains are: the patients, the treatment domain, services provided, and program outcome. Each of these domains will be delineated with respect to data obtained from a three-year comprehensive evaluation of six methadone maintenance programs in three cities. (Figure 1).

The first domain consists of patient data. In the three cities evaluation, patient data was obtained through face-to-face interviews at the programs as well as by record reviews. Repeat interviews with the ASI were obtained in 1986 and a follow-up study of patients who left treatment completed. In general, patient data has not been neglected in contemporary program evaluation.

The second domain in the schema is the treatment domain. This domain includes the setting or environment in which treatment occurs, the treatment policy followed, the leadership and staff who provide services, as well as the physical facility and other resources. In the three cities evaluation, treatment domain data was obtained with respect to 51 variables. This domain of treatment - which includes a major portion of the black box - has been conspicuously neglected in drug abuse treatment evaluation.

The third domain in the evaluation schema encompasses the actual provision of treatment and rehabilitative services. In methadone maintenance programs, this domain includes: number of methadone doses dispensed on site and as take-homes; dosages prescribed; hours of clinic operation; patient attendance; number, type and duration of counseling sessions (e.g., individual or group); educational or vocational services; type and frequency of medical services provided on site; referral services provided; psychiatric coverage provided; frequency of obtaining urine specimens and urinalysis procedure; as well as any other services provided. In the three cities evaluation, data were obtained with respect to 35 service provision variables. This services provided domain has also been neglected in evaluation, but somewhat less so than the treatment domain.

The fourth domain of the schema is patient and program outcome following treatment. The area of patient outcome has not been neglected. Thus, there has been a realization that it is necessary to collect information about patient characteristics following treatment in order to determine whether or not positive change has occurred. In drug abuse treatment evaluation, common outcome criteria are: reduction of drug abuse (by type of drug), reduction of criminality, retention in treatment, improvement in employment and reduction in welfare status. In the three city evaluation, patient outcome was measured by means of ASI scales as well as additional variables.

Conversely, the study of proaram output has been strangely neglected. Although Dr. Dole advocated performance based ratings of programs in 1982, these studies were not undertaken. Therefore, program output remains a largely unexplored area. In the three cities evaluation, program outcome was measured in two ways: (1) the adequacy of the treatment domain and the adequacy of services provided, and (2) differential effectiveness in patient outcome rates.

The present evaluation schema has made it possible to conceptualize, measure and analyze dozens or scores of treatment related variables in each of the four domains. As a consequence, detailed information about patients in methadone maintenance treatment has been obtained; comprehensive knowledge about how methadone clinics are staffed and function has been compiled; the actual provision of medical, counseling and other services enumerated and analyzed; and the effectiveness of individual program according to objective outcome criteria determined.

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AFFILIATION: Addiction Research Center, NIDA,
P.O. Box 5180
Baltimore, Maryland 21224

Figure 1: Treatment Evaluation Schema - Four Domains of Data

	I. PATIENTS	II. TREATMENT DOMAIN	III. SERVICES PROVIDED	IV. OUTCOME
Sample, Scope	Entire Census; Sample of 633	51 Variables	35 Variables	11 Variables
Source of Data	Interviews & Records	Interviews, Records & Observation	Interviews, Records & Observation	From I, II & III
First Measurement	1985	1985	1985	1985
Second Measurement	1986	1986	1986	1986
Place of Measurement*	At Programs	At Programs	At Programs	(Analysis)
Data Collected By:	ISR; MES	ME Staff-4	ME Staff-4	Follow up of 107 by ISR

* Data Collection was at six methadone maintenance programs; two each in New York City, Philadelphia and Baltimore.

Evaluation of Treatment for Cocaine Dependence

Charles P. O'Brien, Arthur Alterman, Dan Walter, Anna Rose Childress and A. T. McLellan

INTRODUCTION

The enormous increase in cocaine availability in the 1980's has presented a completely new problem to the substance abuse treatment community. The clinical picture presented by patients exposed to cheap widely available cocaine is new to all of us. The added complication of a new form, crack. which makes free base cocaine accessible to the most vulnerable of our citizens is a further unpredictable development.

As with any new disease or syndrome, there is a period of time in which clinicians must orient themselves, set up treatment programs based on extrapolation from similar illnesses and on their intuition and then observed the results. Changes and improvements in treatment techniques should be based on feedback from organized studies and preferably from controlled studies with random assignment. Such a deliberate and rational process is unusually difficult when faced with the clinical problems presented by cocaine dependent patients in the late 1980's. Patients apply for treatment while they are in a crisis and often as an acute emergency. Frequently violence or threats of violence are involved. These threats may be either suicidal or homicidal and not infrequently directed toward members of the staff if patients' demands are not immediately met. This air of crisis inhibits a systematic research approach and also interferes with an effective treatment program. It is not surprising, therefore, that there are few studies currently available which describe the results of treatment for cocaine dependence. Rather than review the existing studies, we will briefly present a description of the patient populations, the treatment retention rates and the current status of four treatment studies being conducted at our research center.

POPULATIONS AND TREATMENT PROGRAMS

Table 1 shows the five different populations of cocaine dependent patients. All are veterans who voluntarily applied at the Philadelphia Veterans Medical Center for treatment of cocaine dependence. Over 90% are black in contrast to about 60% black among opiate dependent applicants. A large majority were legally employed just prior to applying for treatment and crime-related activities were relatively small, significantly less than we find among opiate dependent applicants (Table II). The average cocaine dependent applicant had been using

cocaine less than three years and only 11% of the non-methadone population had had prior treatment for substance abuse.

1. *General Program.* This is our basic cocaine treatment program. We currently receive 6-13 new applications for treatment each day and patients are accepted as vacancies occur. The only exclusionary criterion is immediate need for hospitalization, usually because of psychosis. The program consists of five days per week in a multimodality day hospital program which includes group therapy, activities therapy, individual counseling and psychoactive medication as indicated. As as be seen from Table 1, only 37% of patients completed the four week intensive outpatient program. The clearest indicator of early termination from outpatient care is homelessness. One hundred per-cent of homeless applicants have failed to complete the program.

2. *Behavioral Treatment Study.* This outpatient program randomly assigns patients to either psychotherapy or drug counseling with or without a behavioral treatment paradigm which involves extinction of conditioned responses to cocaine related stimuli (Childress et al. 1989). The 51 patients who volunteered for this study were almost all black and 89% were employed prior to beginning treatment. They spent more on cocaine than the other groups and the retention at four weeks for this group was 75%.

TABLE I

FIVE COCAINE DEPENDENT POPULATIONS

	General	Behav.	Methad.	Inpat.	Outpat.
N	121	51	59	25	35
% Black	96	96	90	88	97
Mean Age	34	34	40	33	32
%Employ	82	89	67	64	77
Yrs Ed	12.5	12.5	12	12.4	11.6
Drug cost	$435	$1,500	$770	$412	$508
Yrs Coc	2.8	3	3	2.7	2
4 wk retnt.	37%	76%	78%*	86%	43%

*10 weeks retention in methadone/desipramine treatment

3. *Desipramine for Cocaine Abusing Methadone Patients.* Cocaine is a major drug of abuse among dependent patients receiving methadone treatment. Those with persistent positive urines for cocaine were given the opportunity to participate in a double-blind trial of desipramine or placebo added to methadone treatment. Seventy-eight percent of the 59 patients who volunteered completed 10 weeks of treatment. The results of the trial are reported elsewhere (Arndt, 1988).

TABLE II

Cocaine Dependent vs. Opiate Dependent Outpatients

Legal Problems

	Cocaine Only	Opiate Only	F
N	113	43	
ASI Legal Compos	0.06	0.19	15.05*
Days illegal act	1.4	10.3	40.06*
Money illegal act.	$58	$808	19.27*
Arrests	2.4	9.2	20.71*
Convictions	0.43	5.7	65.66*
Months incarc.	1.7	17.0	30.16*

* p< .001

RANDOMIZED TRIAL OF OUTPATIENT VS. INPATIENT TREATMENT

Populations 4 and 5 of this report participated in randomized trial in which cocaine dependent volunteers were randomly assigned to either a 28 day intensive outpatient program or an inpatient therapeutic community, also of 28 days duration. This study is still in progress, but we will present a preliminary report because of the current need for systematic data on the efficacy of treatment for cocaine dependence.

The research subjects were 60 male cocaine dependent patients. Thirty-five were treated in the four week Day Hospital program of the Philadelphia VA Medical Center and 25 in the 28 day inpatient rehabilitation program of the Coatesville VA Medical Center. These two facilities are in the Philadelphia metropolitan area about 40 miles from each other by car. Of the 35 Day Hospital patients, 26 has been randomly assigned to Day Hospital treatment, while the remaining nine were "non-random" subjects who agreed to participation provided they were treated in the Day Hospital. Of the 25 patients. 16 had agreed to random assignment and by chance received inpatient treatment, while nine participated as non-random subjects who agreed to participation contingent on inpatient treatment. All patients had been screened for participation at the Philadelphia VA Medical Center's Central Intake Unit.

Procedures. All patients underwent both medical and physical examinations, including laboratory evaluations. On this basis, all were determined to be cocaine dependent. This diagnosis was subsequently confirmed by the NIMH Diagnostic Interview Schedule (Robins, et al., 1981). None of the subjects were demented, mentally retarded, currently psychotic, or suffering from an unstable medical condition requiring immediate attention. Informed consent was obtained following full disclosure. The subjects agreed to participation in a study evaluating the relative efficacy and costs of inpatient versus Day Hospital treatment for cocaine dependence. The Day Hospital program consisted of 27 hours of treatment over the five weekdays. Treatment consisted primarily of group therapy supplemented by individual counseling, medical care, family therapy, vocational counseling and recreational therapy. Group therapy concentrated on overcoming denial, helping the patient to structure his life around non-substance related activities and becoming involved in a self-help group. The inpatient treatment was quite similar except for meals and housing and more. structured recreational events.

During the intake process the Addiction Severity Index (ASI - McLellan, et al., 1980, 1985) was administered to all subjects. This instrument obtains historical/sociodemographic information and also assesses level of functioning/problems in seven areas of life functioning: medical, employment, alcohol -related, drug-related, legal, family/social and psychological. The ASI consists of a semi-structured interview which takes a trained research technician about 40 minutes to administer. An abbreviated follow-up version is employed to assess post-treatment functioning. Follow-up evaluations were obtained four months after entry into treatment, i.e. three months after projected completion of the course of treatment.

PRELIMINARY RESULTS OF THE INPATIENT VS OUTPATIENT STUDY

Baseline Findings. Baseline data were analyzed using t-test for continuous data and chi square (Yates correction) for nominal data. In all cases, the data were evaluated both for all subjects (N's =35 and 25) and for randomized subjects only (N's =26 and 17). The Day Hospital and inpatient groups of subjects did not differ in basic sociodemographic characteristics such as age, race, years of education, marital status and socioeconomic class. As was the case with the other three populations we have studied, almost all of the patients were black.

ASI Findings. The data revealed relatively low problem levels for all groups in the medical and legal areas. Moderate problems levels were reported with respect to alcohol and drug problem levels. Thus, the cocaine dependent patients had been using cocaine less than 2.5 years and drinking to intoxication for about 6 years. Negligible heroin use was reported. While the problem levels are certainly serious, the overall drug problem level was lower than that reported for heroin dependent patients at the same facility and the alcohol problem level was lower than that for the alcohol dependent patients we treat. Psychological problem level was also at a moderate level, while reported employment and family social problems were relatively high for these patients.

Treatment Completion. The rates of completion for the month of rehabilitation were 43% for the Day Hospital and 86% for inpatients when all subjects were considered. The difference between the two groups were significant $X2 = 8.72$, d.f. = 1,n = 54, p = .003. Considering the randomized subjects only, the Day Hospital completion rate was 42% while 88% of the randomized inpatients completed treatment. Again, the difference between the two groups was significant: $X2 = 7.02$, d.f. = 1, n = 40,p = .008.

Table III

ASI DATA FOUR MONTHS AFTER BEGINNING TREATMENT

	OUTPATIENT, N=18		INPATIENT, N=15	
	BASELINE	FOLLOWUP	BASELINE	FOLLOWUP
Medical compos.	0.16± .4	0.18± .38	0.19± .33	0.24± .33
Days Med Problems	4.1±9.6	4.4± 9.4	3.1± 8.6	5.4± 8.7
Employ compos.	0.59± .26	0.60± .34	0.66± .23	0.54± .23
Employm. income past 30 (all SS)	337±445	796±1427	244± 303	692± 806
Alcohol compos.	0.17± .20	0.08± .14	0.20± .23	0.12± .19
Days drink past 30	7.1± 6.7	1.3± 1.6	7.4± 8.2	2.6± 4.7
Days intox past 30	5.8± 6.9	0.72± 1.6	6.4± 8.2	1.9± 4.7
Drug compos.	0.25± .06	0.07± .08	0.24± .09	0.13± .10
Days cocaine use past 30 days	10.6± 7.9	0.78± 1.7	9.7± 8.5	1.7± 3.4
Legal compos.	0.06± .13	0.04± .15	0.11± .16	0.00±.01
Days illegal activity	1.2± 3.6	0.00± .00	5.5± 10.7	0.50± 1.9
Fam/soc compos.	0.33± .20	0.18± .22	0.35± .27	0.22± .27
Days family prob	3.0± 7.3	1.8± 3.7	5.3± 8.9	4.00± 8.9
Days problems with friends	0.44± 1.2	0.06± 0.24	3.3± 8.7	0.20± 0.78
Psychol. compos.	0.25± .24	0.18± .27	0.21± .26	0.26± .22
Days psychol prob	5.2± 9.8	5.7± 10.4	7.7± 11.7	9.3± 10.8

Treatment Outcome. A follow-up ASI interview at four months following treatment entry has been obtained on 83% of eligible patients. Seventy-six percent of all patient reported no cocaine use in the prior 30 days and those who were using cocaine were averaging only 1.2 days per month. Urine tests tended to support the patients' statement, but we do not have sufficient urine data yet to report. It should be noted that there were no consequences to reporting drug use to the follow-up interviewer. ASI data were obtained on 18 patients (14 randomized) and 15 patients (nine randomized). The data were evaluated utilizing one way analyses of variance with repeated measures. There was a significant effect for time for all groups. inpatient, outpatient, randomized and non-randomized. Differences between baseline and follow-up were significantly improved at $p<.04$ or better for all ASI factors except Medical and Psychological variables. There were no differences between those patients who had received inpatient treatment and those who had received outpatient treatment despite the much higher treatment completion rate for the inpatients.

SUMMARY

1. At the Philadelphia VA Medical Center, veterans applying for treatment of cocaine dependence were significantly different from applicants dependent on opiates or alcohol without cocaine. The cocaine dependent patients were almost all black and they were mostly employed. Few were involved in crime and, for most, this was the first course of substance abuse treatment. Their use of cocaine began within the past three years and they had relatively few psychiatric, medical, employment or family problems when compared with other applicants for substance abuse treatment.

2. Completion rate for a course of outpatient treatment for cocaine dependence varies from a low of 37% in the general Day Treatment Program to 76% in a special Behavioral Treatment Program and 78% among methadone patients receiving an additional medication for cocaine dependence. Completion of a 28 day course of inpatient treatment was 86% whether the patient selected inpatient care or was assigned to it on a random basis.

3. Those outpatients who remained in treatment generally did well and refrained from cocaine use even during the course of outpatient, either day hospital treatment or behavioral treatment. When both inpatients and outpatients were re-examined four months after beginning treatment, there was significant improvement on almost all ASI categories. Both drop-outs and completers were re-examined. Despite the significantly greater completion rate for the patients assigned to inpatient treatment, both groups showed equal levels of improvements at the 4-month follow up point.

4. These preliminary results from five different treatment populations suggest that cocaine dependent patients can be engaged in treatment and that significant improvement is possible.

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ACKNOWLEDGEMENTS

This work was supported by USPHS Grant DA 05186 and by the VA Medical Research Service.

AUTHORS

Charles P. O'Brien, MD, Arthur Alterman. PhD, Dan Walter, MA, Anna Rose Childress, PhD, and A.T. McLellan, PhD.
Addiction Research Center, University of Pennsylvania and Philadelphia VA Medical Center, 3910 Chestnut Street, Philadelphia. PA 19104

Current Status of Alcoholism Treatment Outcome Research

Richard Fuller

This review is selective rather than comprehensive. I have chosen those areas which I consider the most important, and in those areas have selected either the papers which I consider the most important or illustrative of the area. The views expressed are mine and not those of the National Institute on Alcohol Abuse and Alcoholism.

Alcoholism treatment has developed mostly from three sources: 1) the experience of recovering people and clinicians who had worked for years with recovering alcoholics 2) pharmacotherapeutic research, and 3) behavioral therapy research. The class example of the first source is the prototype self-help movement, Alcoholics Anonymous (AA). Alcoholics Anonymous developed before alcoholism treatment was an active area of research, and, thus to a large degree, its efficacy not been evaluated by randomized controlled clinical trials (RCTs). This situation is similar to accepted pharmacotherapies such as the antiarrhythmic medications, quinidine and procainamide, which became accepted into medical practice before clinical trial methodology was developed. Once a treatment becomes accepted, it is difficult to do a RCT to test its efficacy because of the ethical dilemma of depriving half of the patients of a treatment perceived as effective.

Much of what is done in alcoholism treatment appears effective, and health utilization data discussed below supports this. Yet, for the specific components of standard treatment we often lack compelling data that the changes in behavior seen are, in fact, the result of the treatment. In contrast, pharmacotherapies and behavioral therapies were usually developed in academic settings and their efficacy has been tested by RCTs. (This is not entirely true for disulfiram which was initially evaluated by uncontrolled studies, and only more recently evaluated by RCTs). Our knowledge of the effectiveness of two of the pharmacotherapies, disulfiram and lithium, is enhanced because not only large scale multi-site studies with sufficient sample size to assure acceptable statistical power. While some behavioral therapies have been found to be effective, their generalizibility is limited because the sample sizes were modest and the benefit may occur only in a special subtype of alcohol dependent person recruited into those studies.

From a broad perspective, alcoholism treatment is effective. Holder and Blose (1986) studied the effect of alcoholism treatment on health care utilization and costs of federal employees in a health insurance program provided by the Aetna

Insurance Company. In the years before treatment, alcoholics incurred gradually increasing health care costs. These costs increased markedly (two and one-half times) in the six months before treatment. The costs declined during the year after treatment and remained stable for the next two years (the period of data collection). For individuals under 45, costs declined to a point comparable to the lowest pretreatment levels. This is an important study and strongly indicates that what is done under the rubric of alcohol treatment is effective. A skeptic can argue that without a concurrent control group one cannot be sure that is was spontaneous remission rather than treatment which was responsible for this happy outcome. However, the temporal linkage is so strong that this possibility seems unlikely. This study does not, however, settle the issue of what components in the treatment package are critical and which are unnecessary.

The most common approach to the inpatient treatment of alcohol dependence is the "Minnesota Model" or some variation of it. It is called The Minnesota Model because it evolved from three treatment centers in Minnesota: Pioneer House, Hazelden and Willmar State Hospital, which were founded in the late 1940s and early 1950s. In its simplest form, treatment consists of assessment and admission to a residential treatment facility for 3-6 weeks followed by intensive attendance at AA (Cook, 1988a). The inpatient phase is intensive, offering counseling, lectures, and group therapy. The content is based on the principles of AA and the goal is abstinence. The staff includes both recovering persons and professionals.

Post-treatment surveys have been done on patients treated at Hazelden (Gilmore, 1985). It is difficult to accept the results of these surveys at face value because of several serious methodological problems. In the only survey to cover a full one year period almost half of the questionnaires were either not sent or not returned. Cook (1988b) recalculated the outcome results by including patients unaccounted for. His calculations indicated a "good outcome" for 63%. However, as Cook points out, this figure is probably an exaggeration because of lack of clearly defined outcome criteria. Furthermore, the surveys relied on self-report and the use of self-report alone to evaluate alcohol treatment outcome research results in inflated outcome results (Fuller et al., 1988). Additional methodological problems of these studies include lack of control or comparison groups and need for longer follow-up (Cook 1988b).

Lectures are one component of the Minnesota Model. Do they add benefit to the other components of the model? This question has not been answered. However, whether patients retain the material they have been taught has been studied. Two different teams of investigators (Sanchez-Craig and Walker, 1982 and Becker and Jaffee 1984) found that alcoholics tested 3 weeks after beginning abstinence were unable to recall treatment-related information presented in a film and, thus, unlikely to influence post-treatment behavior.

While this questions the therapeutic value of one part of the Minnesota Model, a more important question is how effective is AA, the central core of the Minnesota Model. Most studies of AA are correlational in nature and support a relationship between attendance at AA and abstinence. Only a few controlled evaluations of AA persons have been done and these were in court-mandated programs (Brandesma, et al., 1980, Ditman et al., 1967) or with patients in a methadone maintenance programs (Stimmel et al., 1983). Whether the results of these studies are generalizable to other alcoholics is unknown. Because of

AA's central role in so much of alcoholism treatment, it is unfortunate that more controlled studies of it have not been done. It reflects the problem of trying to study a treatment after it has become firmly established in practice. The Holder and Blose (1986) data indicate the alcoholism treatment does work. What is missing is more specific information about the benefit of the various activities done under the table alcoholism treatment.

An effective pharmacological treatment for alcoholism would be of great benefit. Vincent Dole (personal communication) was told by Bill W., one of the co-founders of AA, that what alcoholism treatment needed was a methadone equivalent, i.e. a medication that would abolish craving. Unfortunately, alcohol does not act on a specific receptor as the opioids do and a magic bullet for alcoholism treatment is not currently available. Nevertheless, research is being done on development of "anti-craving" drugs.

Disulfiram is the most widely used medication, specifically used to treat alcoholism. It causes an adverse reaction if a person drinks alcohol, and thus, is a deterrent to drinking. The initial studies reported excellent results, but these studies had serious methodological flaws. Only 10% of the studies were controlled. The studies were not "blinded", relied on self-report only, and the therapists were the ones assessing the response to treatment. The recently published multi-site VA cooperative disulfiiam study was a randomized controlled, blinded clinical trial of 605 men (Fuller, et al., 1986). There were two control groups: a 1 mg disulfiram dose and a no-disulfiram group. One mg is not sufficient to cause disulfiram-ethanol reaction (DER) and this group was a control for the threat of a DER. The no-disulfiram group was a control for the counseling all the patients received. There were no significant differences in the proportion of men achieving continuous abstinence for one year among the disulfiram group (18.8%) and two control groups (22.5% and 16.1%. respectively) nor in time to first drink among the three groups.

There was a highly significant relationship between compliance to regimen and continuous abstinence. Forty-three percent (50/1165) of the men judged to be compliant were abstinent compared to only 8% (26/315) of the non compliant men. This significant relationship was true for all three treatment groups, suggesting that something occurred in these men's lives to promote both compliance and abstinence or vice versa.

Among the men who relapses and who provided all seven treatment response assessment interviews (about half of the men who relapsed), there were significantly fewer drinking days during the year (49.0 + 8.4) reported by the 250 mg dose disulfiram patients compared to the two control groups (75.4 + 11.9) and 86.5 + 13.6. respectively). This significant increase in abstinent days in the conventional disulfiram dose patients was corroborated by a cohabiting relative or friends. The men who provided all assessments interviews were slightly but significantly older and had lived significantly longer at their current address than those who provided less than 11 follow-up interviews. Abstinence is difficult to achieve for any addiction and these findings suggest that disulfiram may be partially beneficial, i.e. reduce the frequency of drinking in men who can not remain abstinent.

Faithful compliance with the medication regimen was the Achilles's heel for many men in the VA study, only 20% were compliant. One study (Azrin et al, 1982) of 43 men attending a rural outpatient clinic found significantly more

abstinent days in men whose spouses supervised the administration of disulfiram than those who were given it to administer themselves. Another recent study (Sereny et al. 1986) has indicated that if supervised disulfiram (in the report administered by the staff) can be mandated as part of the treatment, this will result in greater rates of abstinence among clinic attenders who previously had failed the treatment. These methods of giving disulfiram deserve more study because they are likely to increase compliance. Disulfiram tablets have been implanted subcutaneously to assure compliance. Unfortunately, the DER is weak or non-existent and a recent controlled study showed no benefit from implanted disulfiram (Johnson et al., 1987). Work is in progress to develop an injectable form of disulfiram,

Calcium carbamide is another deterrent drug with a short duration of action (12 hours). It is available in Canada, but not in the United States and there have been no controlled trails assessing its efficacy.

Lithium carbonate is another medication which held promise of being an effective treatment. Both human data (Judd and Huey. 1984) and animal data (Ho and Tsai, 1976) indicated reduced craving for alcohol and/or reduced consumption. Fawcett et al. (1987) did a randomized controlled blinded study of lithium carbonate in 104 alcoholics. Overall, there was no difference in resumption of drinking between patients receiving lithium and those receiving placebo except for the compliant patients. The compliant lithium carbonate patients were significantly less likely to resume drinking than were the placebo patients. However, compliance was not determined by the same method in both groups. Compliance in the lithium carbonate group was measured by blood levels and in the placebo group by pill counts.

The results of VA cooperative study of lithium carbonate (Dorus et al) have been presented at meetings and the results arc soon to be published. This study of alcoholics with and without depression found no benefit from lithium carbonate in either type of alcoholic; and no differences were found between patients complaint with the lithium carbonate or the placebo regimen. Because of the sample size (286 non-depressed alcoholics and 171 compliance in both active drug and placebo groups. this study appears to provide the definitive answer about the efficacy of lithium carbonate.

Disulfiram and lithium carbonate have been the most extensively studied of the pharmacological treatments. The serotonin uptake inhibitors are currently the most actively investigated medications. These agents block the presynaptic uptake of serotonin, and, thus, increase the concentration of serotonin at the synapse. Interest in these blockers derives from animal studies showing a negative correlation between brain serotonin levels and spontaneous alcohol consumption and more than a dozen animal studies showing that specific serotonin uptake blockers decrease alcohol preference and intake in rodents (Gorelick, 1989). furthermore, several studies have found significantly lower levels of cerebrospinal fluid 5-hydroxyindol acetic acid (5-HIAAA), the major metabolite of serotonin in alcoholics both during acute withdrawal and abstinent for at least 5 days and a negative correlation between length of abstinence and the cerebrospinal 5-HIAAA levels (Gorelick, 1989).

Four double-blind, placebo-controlled human studies, using different serotonin uptake blockers have been reported. While all found a reduction in alcohol intake, only one study was done with alcohol dependent subjects (Gorelick,

1986). The others recruited "social drinkers" and "early stage problem drinkers". Gorelick studied 10 subjects received either fluoxetine (up to 80 mg orally daily) or placebo and could request alcohol according to a fixed interval schedule, i.e. 60 ml f 97.5 proof alcohol once an hour 13 times daily. During the first week fluoxetine caused a significant reduction of daily alcohol intake from 317 ml to 273 ml (a 14% reduction) and a significant decrease in alcohol craving as rated by nurses. However, this effect lasted only one week. There were no significant changes during the remainder of the study.

While these medications have been shown to decrease alcohol consumption, the decrease is usually modest (about 10%) and the effect was quite transient in Gorelick's study. These factors limit the potential benefit of these medications unless one of the other members of this class of agents (e.g. fluovaxamine) are shown to be more potent and long lasting or the chemical structure of one of these medications can be altered to enhance its effect

Other pharmacological agents e.g. naltrexone and bromocriptine are being studied, but the data either are too preliminary and are not available to reach any conclusions, Agents like emetine have been used to create an aversive conditioning response to alcohol. This literature is characterized by a lack of controlled clinical trials (Wilson, 1987). Short-term conditioned aversion reactions to alcohol can be produced, but the efficacy of chemical aversion conditioning independents from standard treatment has not been demonstrated (Wilson, 1987).

The other major category of therapies studied are the behavioral therapies which are derived from three theoretical psychological models: classical conditioning, operant conditioning, and social learning theory. Most of the controlled studies of alcoholism treatments have been of behavioral approaches. However, many of the studies have recruited subjects who are not alcohol dependent and the sample sizes of the studies are often small (less than 60). Behavioral therapies applied to alcoholism treatment include aversion therapy, cue exposure, community reinforcement, self-control training, stress management, social skills training, marital behavioral therapy and relapse prevention. Aversion therapy is an example of a classical conditioning response that attempts to produce a conditioned aversion response to alcohol by pairing an aversive stimulus with the sight, smell or taste of alcohol. Chemical aversion therapy using emetine has been discussed above.

Another example of classical conditioning is based on extinction training. Cue exposure involves presenting the patient with stimuli associated with alcohol consumption but preventing him from drinking. Eventually the patient reports less craving for alcohol and during trials manifests attenuated autonomic reactions to alcohol cues. The ultimate value of such diminished responses has yet to be demonstrated.

Community reinforcement is based primarily on principles of operant conditioning. Community reinforcement involves enhances access to reinforcing activities, e.g. family, non-drinking social club, and work, and makes access to these reinforcers contingent on abstinence. Azrin et al. (1982) have combined community reinforcement and disulfiram. They have reported that this combination resulted in almost complete abstinence during a six month study contracted with patients assigned to "traditional;" therapy drinking 50% of the time by the sixth month. These are remarkable results but the sample

consisted of 43 men seen at a rural Illinois outpatient clinic. Currently controlled studies are being done in the United States and Poland to corroborate or refute these results.

Marital therapy is relevant because post-treatment research has (Moos and Moos, 1984) shown a strong relationship between favorable family adjustment and sustained abstinence. McCrady and associated (1986) compared three out-patient treatment conditions: 1) minimal spouse involvement; 2) alcohol-focused spouse involvement; and 3) alcohol focused spouse involvement plus behavioral marital therapy. At six months there were no significant differences among the three groups in either total abstinence of number or abstinent days, although all subjects had markedly decreased their drinking. However, the marital therapy subjects decreased their drinking more quickly, relapses more slowly, and maintained their marital satisfaction better after treatment. O'Farrell et al (1985) also found better marital functioning in those receiving behavioral marital therapy and significantly more alcohol-free days in the marital behavior group members.

Relapse prevention strategies rely on cognitive therapy techniques to avert post-treatment relapses. Marlat (1989) has presented data of a controlled trial in "problem drinkers," showing fewer relapses and drinking days n the drinkers receiving relapse prevention training. Whether this method is effective in alcohol-dependent individuals, remains to be demonstrated.

Conclusion: Alcohol treatment works as measured by a reduction in health care utilization. However, what specifically under the rubric of alcoholism treatment works is, for the most part, not known because adequate controlled studies have not been done. Disulfiram appears to increase abstinent days in men with families and residential stability. Some behavioral therapies appear promising, but have been studied in small samples and often in non-dependent alcohol abusers so that generalizability is unknown. We need new innovative treatment methods, a better understanding of current methods, and learn how to enhance treatment by matching treatments to specific patient subtypes.

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AUTHOR

Richard K. Fuller, National Institute of Alcohol Abuse and Alcoholism, Rockville, MD

Pain Modulation: Opiates and Chronic Pain

Howard L. Fields

INTRODUCTION

Persistent and/or frequently recurrent pain is a problem that afflicts millions of people. For example, in a recent survey, 8% of a random sample of the population of the United States reported back pain severe enough to limit their activities for at least 30 days (Taylor and Curran 1985). This translates into about 20 million people losing 500 million work days from back pain alone. Thus, the cost, both in terms of human suffering and economic loss is staggering. Patients with arthritis, cancer pain, severe headache and low back pain provide us with a serious challenge to elucidate the neural mechanisms that mediate the pain and to devise better ways to treat it. Over the past two decades the related fields of pain research and clinical treatment have begun to address these objectives. In this paper, I will briefly review some of the significant advances that have contributed to our understanding of chronic pain. I will also discuss recent work on the mechanisms of opiate analgesia that may help us to understand some chronic pain patients.

TRANSMISSION

The transmission of activity evoked by noxious stimuli begins with the transduction process in the peripheral terminals of the primary afferent nociceptor (PANs). The message is conducted to pain transmission neurons in the spinal cord dorsal horn and from there to thalamus and cortex. In human subjects, PAN discharge and subjective pain intensity covary across the same range of stimulus intensities (Gybels et al., 1979). Across this same range of stimulus intensities primate spinothalamic tract neurons and human subjective pain ratings also covary (LaMotte and Campbell 1978; Price et al., 1978). A similar stimulus intensity/discharge frequency relationship has been demonstrated for neurons at thalamic and cortical levels (Kenshalo et al., 1980; Kenshalo and Isensee 1983). These

studies indicate that stimulus intensity is encoded in a direct manner by neuronal discharge frequency at each level of the pain transmission system. With this simple code, the mechanisms for persistent pain can be understood by a single unifying principle: any process which leads to prolonged increases in activity in PANs or central pain transmission neurons can contribute to persistent pain.

There are several distinct neural processes that have the potential to produce pain or to increase its duration and/or intensity. The most straightforward cause of persistent pain is a persistent, active tissue-damaging process (e.g. cancer, arthritis or a herniated intervertebral disc). In these cases the pain will persist unless the underlying disease is cured. This possibile cause for chronic pain is the one most often recognized and correctly managed by physicians.

Sensitization. In some cases of persistent pain no active tissue damaging process can be identified. The patient may point to an area that they say is painful but nothing can be found that would be expected to activate a primary afferent nociceptor. A theoretical possibility in such cases is that primary afferent nociceptors have been sensitized so that intense stimuli are no longer required to activate them. Thus, normally innocuous stimuli such as brushing the skin or weight bearing on joints can provoke pain. In fact, sensitization of primary afferent nociceptors (PANs) does occur under 'physiological' conditions (LaMotte et al., 1983). Peripheral inflammatory mediators such as histamine, prostaglandins and leukotrienes can sensitize PANs (Lembeck 1983; Levine et al., 1984). Furthermore, there is now good evidence that peptide neuromodulators (e.g. substance P), are released from the peripheral terminals of PANs and contribute to their own sensitization (Juan and Lembeck 1974).

Sympathetic Efferents. Activation or sensitization of PANs may be produced by activity of sympathetic efferents. Certain patients have a burning pain that can be completely relieved by blockade of sympathetic efferents. Such patients are said to have sympathetically maintained pain (reflex sympathetic dystrophy (De Takats 1937), or causalgia, if there is accompanying nerve damage (Mitchell 1965). Whether sympathetic efferents can activate or sensitize PANs is still an open question (Roberts 1986). however, there is evidence for sympathetic efferent induced peripheral release of prostaglandins which can sensitize some PANs (Gonzales et al., 1989). It should be pointed out that experimental studies of sympathetically maintained pain have focussed on cutaneous PANs. However, the innervation of the skin is quite unique and chronic cutaneous pain is rare. Studies of other PANs may be required to elucidate the mechanism of sympathetically maintained pains.

Muscle Spasm. The investigation of sensitization of deep receptors, particularly musculoskeletal PANs, is needed to understand muscle pain which seems to be extremely common in patients referred to chronic pain management centers (Fishbain et al., 1986). Such patients have muscles with focal areas of contraction and tenderness (myofascial pain syndrome (Travell and Simons 1983)). The pathology and cause of this 'syndrome' is unknown (some even question the validity of the concept). However, many of these patients obtain dramatic pain relief with stretching or with injections of small amounts of local anesthetic into tender-points of the involved muscles. This observation implicates muscle PANs.

Neuropathic Pain. Persistent pain is occasionally produced by injury of either the peripheral or central nervous system. Damage to the axons of PANs can result in the generation of spontaneous action potentials from sites along the damaged axon (Devor 1984; Howe et al., 1977). Such ectopic impulses would be interpreted as pain by the patient. Spontaneous discharge can also be reliably observed in central pain transmission neurons following damage to their afferent input (Lombard and Larabi 1983)

Prolonged Effects of Afferent Input. In addition to the sensitization of PANs by noxious stimulation or sympathetic nervous system activity, there is good evidence that noxious stimulation in the periphery can induce very long lasting changes in spinal cord neurons. For example, a brief burst of impulses from muscle PANs can enhance spinally mediated withdrawal reflexes for hours (Woolf and Wall 1986). Whether such long lasting changes in the spinal cord contribute to any clinical chronic pain syndrome is unknown, however, some recent clinical studies are consistent with this possibility. In thes studies, patients who were premedicated with opiate analgesics or local anesthetic block to reduce preoperative or intraoperative pain had less post-operative pain (McQuay et al., 1988; Bach et al., 1988).

PAIN MODULATION

In the discussion to this point I have considered only processes that are subsumed under the general category of pain transmission. However, it is clear that central pain transmission neurons are under the control of powerful modulating networks, some of which have endogenous opioid peptide component neurons.

The discovery of these networks, and of the endogenous opioid peptides that contribute to its action, are among the most dramatic breakthroughs in the understanding of pain.

Although it had been appreciated for many years that complex brain pathways modulate sensory transmission, the presence of a selective pathway for controlling pain was first clearly demonstrated by the discovery of stimulation-produced analgesia (SPA). Reynolds, in 1969, showed that electrical stimulation in the midbrain area of the rat produces an analgesic effect. During stimulation, the rats gave no evidence of being disturbed by noxious stimuli. However, they were able to move and to orient to visual and auditory stimuli. When the brain stimulation was stopped, noxious stimulation produced a variety of pain-associated behaviors: attempts to escape, vocalization, biting, and signs of anxiety. In an attempt to apply this observation clinically, several groups of neurosurgeons placed electrodes in that same regions of the human brain, the midbrain periaqueductal gray matter (Hosobuchi et al., 1977; Richardson and Akil 1977). These surgeons demonstrated an analgesic phenomenon apparently similar to that reported in the rat: over a period of approximately 10 to 15 minutes, patients experienced a fading of pain. The demonstration of SPA in humans firmly established the existence of a selective pain suppression system.

Subsequent animal studies have shown that SPA can also be obtained from discrete sites in the medulla. Anatomical studies have demonstrated that there is a discrete pathway from the midbrain to the medullary SPA sites and from the medulla to the dorsal horn of the spinal cord and trigeminal nucleus caudalis (Basbaum and Fields 1984).

Opioid Peptides. The endogenous opioid peptides are found in the periaqueductal gray and medullary analgesia region, and in the spinal cord. Thus, there is a striking parallel between the distribution of endogenous opioid peptides and the sites that, when electrically stimulated, produce analgesia. In addition, microinjection of opiates at these same sites produces analgesia, indicating that morphine produces analgesia by mimicking the effect of opioid peptides.

As mentioned above, the modulation network projects to the spinal cord, where it inhibits pain-transmission neurons. The brainstem cells that project to the spinal cord and inhibit pain transmission are concentrated in two regions, the rostral ventromedial medulla, which is rich in serotonin, and the dorsolateral pons, which is rich in norepinephrine. Electrical stimulation in either region inhibits nociceptive spinal neurons and produces behavioral analgesia.

Activation of the Analgesia System. Research to date indicates that stress and pain are both major activating factors (Basbaum and Fields 1984). In man, stress may be produced by anxiety or by any extreme emotional state. Assuming endogenous opioid involvement, the activity of the analgesia system can be demonstrated by giving naloxone, a narcotic antagonist, to

subjects who have not received exogenous opiates. In normal, pain-free individuals, naloxone itself has no effect. However, naloxone increases the reported intensity of postoperative dental pain, indicating that the stress and pain of the surgery is sufficient to activate the analgesia system (Levine et al, 1978; Gracely et al., 1983).

Another approach to determining the physiological function of pain modulation networks to monitor the activity of its constituent neurons. To this end we have recorded from neurons in the rostral ventromedial medulla (RVM) (Fields et al., 1988). This area, which includes the nucleus raphe magnus, is a convenient area to study using neurophysiological methods. A high percentage of its neurons have activity that is influenced by noxious stimulation and project to the spinal cord dorsal horn.

The study of RVM neurons has provided us with unexpected insights into the functional circuitry underlying pain modulation. Most of our studies have been carried out in rats under light barbiturate anesthesia. Radiant thermal stimuli are delivered to the skin under feedback control and single unit activity is monitored using extracellular recording techniques. The time of onset of escape reflexes is also monitored using a force transducer. With these techniques we have defined three classes of neuron in the RVM: on-cells whose discharge increases abruptly just prior to withdrawal from noxious heat; off-cells, whose activity decreases just prior to withdrawal; and neutral cells, whose activity is not correlated with noxious stimulation or withdrawal. These neurons are intermixed in RVM and a similar percentage of each class can be shown to project to the spinal cord (Vanegas et al., 1984). Although it is presently unclear what, if anything, neutral cells contribute, there is compelling evidence that both on and off cells modulate nociceptive transmission.

Simultaneous recording from both on and off cells in RVM under light barbiturate anesthesia reveals that they are active during alternating periods. Thus the RVM alternates between periods when on-cells are active and periods when off-cells are active (Barbaro et al., 1989). Withdrawal reflex latency is shorter when on-cells are active, longer when off-cells are active (Heinricher et al., 1989). When morphine is given, off-cells fire continuously, while on-cells become silent, both effects being reversed by naloxone. Neutral cell activity is unaffected by morphine. Since off-cells are the only cell class whose discharge is increased by morphine and since lesions of RVM or its projection route to the spinal cord block opiate analgesia, it is clear that off-cells are RVM inhibitory output neurons.

Nociceptive Facilitation. The more intriguing question that arises from these studies is that of the function of the on-

cell. Its discharge pattern and the fact that, with most manipulations, its discharge changes in a reciprocal way to that of off cells suggest that the on-cell facilitates nociceptive transmission. Direct support for this concept comes from pharmacological studies. First, alpha-l adrenergic agonists excite on-cells when iontophoresed nearby and alpha-2 adrenergic agonists have an inhibitory action (Heinricher et al., 1988). Significantly, adrenergic agonists have no direct effect on off-cells. The functional importance of these studies is indicated by microinjection of drugs into RVM; alpha-l agonists facilitate and alpha-2 agonists inhibit spinally mediated nocifensor reflexes (Sagen et al., 1985).

Further experimental evidence that on-cells facilitate nociception comes from studies of acute physical dependence in rats. In lightly anesthetized rats when naloxone is given after a single dose of morphine, an abstinence syndrome is produced. One of the most reliable features of this abstinence syndrome is a shortening of nocifensor reflex latency. During this period of hyperresponsiveness on-cells fire at rates that are much higher than they were in the pre-morphine baseline period (Bederson et al, 1987). In fact, the magnitude of the hyperresponsiveness is directly correlated with the increase in on-cell discharge. Since off-cells are not firing during this period, this data is consistent with the idea that the on-cell has a direct facilitating effect on nociceptive transmission.

The notion that a central modulatory network can both suppress and facilitate pain transmission is an important conceptual advance. If, as is generally accepted at present, pain modulation were a unidirectional inhibitory process, activity of the network could only be manifested at the behavioral level in the presence of noxious input. By having a facilitatory component it becomes possible, with the nervous system intact, to generate a pain signal in the absence of a noxious stimulus in the periphery. Thus activity in on-cells could drive dorsal horn nociceptive transmission neurons. It is not clear whether an-cell driving of dorsal horn neurons could produce a specific, well-localized somatic pain. However, because pain intensity is encoded by discharge frequency, it is easy to see how on-cell activity, through a generalized excitatory effect on nociceptive transmission neurons, could increase the perceived pain intensity produced by any stimulus.

These studies also suggest a potential link between chronic pain and opiate dependence. If there were individuals with relatively greater on-cell firing in any given situation, they might experience a given noxious input as more intense. Similarly, intense but not frankly noxious stimuli might be experienced as painful. Such individuals might also have a relatively greater on-cell discharge with falling plasma opiate levels and thus experience abstinence as more unpleasant. Along these lines, it is interesting that low back pain has

recently been shown to be a reliable measure of naloxone induced opiate abstinence in ex-addicts (Heishman et al., 1989). Thus, the distinction between patients requesting drugs for pain and those who need drugs to avoid abstinence may be somewhat artificial. It also raises the possibility that some groups of patients with chronic pain may be at relatively higher risk for opiate dependence. At the very least, opiates, by inducing dependence, could play a role in perpetuating pain.

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ACKNOWLEDGEMENTS

Supported by a grant from the National Institute on Drug Abuse, DA-01949, and the Bristol-Myers Foundation.

AUTHOR

Howard L. Fields, M.D., Ph.D.
Departments of Neurology and Physiology
University of California, San Francisco
San Francisco, CA 94143

Clinical Effectiveness of Analgesics in Chronic Pain States

Harlan F. Hill and C. Richard Chapman

INTRODUCTION

In recent years the problem of chronic pain has been recognized and its impact on society and the health care system has captured the attention of both health care providers and administrators, We now know that conventional medical methods for managing acute pain are inappropriate therapies for many chronic pain patients. Surgical and pharmacologic solutions to pain problems have largely given way to behavioral and rehabilitation approaches. This paper briefly reviews the present and potential contributions of pharmacology to the comprehensive, multidisciplinary treatment of chronic pain. We argue that pharmacologic therapy is an underused resource. Careful consideration of the varieties of chronic pain and review of a largely overlooked literature on opioid management of certain chronic pains suggests that selective application of analgesic drugs can be of substantial value in the management of certain chronic pain states.

Although we recognize the profound influence of behavioral factors in the chronic pain problems seen in major referral clinics, we argue here that many of the chronic pains seen in conventional medical practice do, in fact, exist because of persisting nociception; still others originate in abnormal neurologic function. It is the responsibility of the physician to pursue the possibility of nociception or neuropathology assiduously and not simply to "rule it out." When the cause of pain can be defined, pharmacologic therapies are major elements in the physician's therapeutic armamentarium.

Types of Chronic pain. states can be divided conveniently and usefully into three broad categories. First, certain chronic pains are based on ongoing nociception. In these pains, the nervous system is intact and tissue damage is the persistent stimulus. Examples of chronic nociceptive pain are arthritis, cancer pain, arachnoiditis and myofascial pain. Then, there are pains that arise from damage to the central or peripheral nervous system; chronic neuropathic pain such as reflex sympathetic dystrophy, postherpetic and trigeminal neuralgias, diabetic

neuropathy, post-stroke pain, phantom limb pain, and many others. Finally, many cases of chronic pain exist for which an underlying cause or pathophysiology cannot be found; or, the pain behavior greatly exceeds that expected for the evident pathophysiology. These so-called idiopathic pains defy medical treatment and sometimes arise from it.

Most chronic pain patients present with one of the three categories mentioned above. It is important to distinguish patients with neuropathic pain from other pain patients because the drugs that relieve neuropathic pain are not the conventional analgesics that modulate pain of nociceptive origin. Appropriate treatment must address the specific type(s) of pain involved in the patient's complaint. Furthermore, many patients have more than one type of chronic pain. This is especially true of cancer patients who frequently have chronic nociceptive pain, chronic neuropathic pain and idiopathic pain simultaneously (Foley 1985). Successful treatment of the neuropathy only or nociceptive pain alone will still leave behind a chronic pain patient.

NEUROPATHIC PAIN

Certain drugs that do not modulate nociception can relieve or reduce non-nociceptive, neuropathic pain; such drugs have been referred to as atypical analgesics. Their pain relieving actions are pain mechanism specific. A prime example of this is seen with reflex sympathetic dystrophy syndrome (RSDS). RSDS is an excruciatingly painful condition resulting from injury to peripheral nerve and involving sensitization of nociceptive nerve endings to norepinephrine released from sympathetic postganglionic terminals. Knowing the pathophysiology allows selection of adequate therapy and pain relief even though the drugs employed (guanethidine or local anesthetics) are not usually viewed as analgesics. In trigeminal neuralgia, nociceptive axons in one or more trigeminal branches undergo focal demyelination and discharge spontaneously (Burchiel 1980); drugs most commonly used as anticonvulsants (carbamazepine, phenytoin, clonazepam) are very useful as "analgesics" under these well-defined conditions. They act by limiting the generation of impulses at ectopic sites on primary afferents (Fields 1987).

Use of tricyclic antidepressants in chronic pain states continues to be an area of active research. The ability of drugs such as amitriptyline and doxepin to relieve pain of postherpetic neuralgia (Max et al., 1988) and diabetic neuropathy (Max et al., 1987) has been further documented in several well-controlled studies. Tricyclic antidepressants are effective in these chronic pain states at lower doses and in shorter times than required for their antidepressant effects. Their effectiveness in other disorders such as post-stroke pain (Leijon and Boivie 1989) opens the possibility that tricyclics may ameliorate a variety of deafferentation pains in addition to their widely known benefits. The mechanisms responsible for the analgesic effects of tricyclic antidepressants remains unclear, although they do not seem to depend on either the relief of depression or the actions of serotonin directly (Kishore-Kumar et

al., 1989). Tricyclic antidepressants may relieve pain by blocking reuptake of both norepinephrine and serotonin in the central nervous system; blockade of serotonin reuptake alone is not sufficient.

Locally applied steroids and local anesthetics are also useful for certain chronic pains. Epidural steroids effectively relieve the persistent inflammation and pain of arachnoiditis. Local anesthetics can produce highly localized sympathetic blockade which alleviates pain in reflex sympathetic dystrophy, and they are effective in many neuralgias (especially lidocaine) for suppressing ectopic neuronal firing (Lindstrom and Lindblom 1987). Lidocaine infusion can relieve pain of diabetic neuropathy for as long as 21 days after a single 30-minute infusion (Kastrup et al., 1987). The most resistant neuropathic pains are those produced by deafferentation. In such cases, pain arises centrally due to altered neuronal dynamics in the spinal cord and/or brain (Lombard and Larabi 1983; Fields 1987). The central mechanisms underlying deafferentation pain remain mysterious and there is no generally accepted, adequate treatment although carbamazepine, tricyclic antidepressants and even opioids have been reported to be beneficial (Urban et al., 1986).

NOCICEPTIVE PAIN

Conventional analgesics act by modulating generation or central transmission of nociceptive information. Nonsteroidal antiinflammatory drugs (NSAIDs) can modulate nociceptive impulse generation by inhibiting production of prostaglandins and related products in peripheral tissue. Opioids act at many levels of the central nervous system (CNS) to decrease nociceptive neurotransmission. Conventional analgesics are most useful in chronic pain states maintained by nociceptive processes. Chronic pain from rheumatoid arthritis and cancer (inflammatory components) respond to NSAIDs. Opioids alleviate such pains as well; however in practice they are usually reserved for the chronic pain of cancer. Cancer pain usually has a distinct nociceptive component, stimulation of normal nociceptors by ongoing tissue damage, which *is* the sine qua non for opioid treatment. Second, cancer patients are expected to have a shortened life expectancy, so the complicating issue of physical dependence on opioids is of less concern. We address this issue below.

In rheumatoid arthritis, opioids may decrease pain effectively, but they do not affect joint destruction and inflammation and may obscure the need for antiinflammatory drugs (Carruthers 1980). Gout is another example of a painful condition maintained by ongoing nociception; its treatment with colchicine and uricosuric drugs is quite specific for the underlying pathology. Finally, the pain of migraine and tension headache are effectively treated with ergotamine and tricyclic antidepressants, respectively (Couch and Hassanein 1979). So even the nociceptive chronic pains cannot be approached singularly with respect to adequate pharmacological treatment for pain reduction; optimal treatment depends on an understanding of the underlying pathophysiology.

We often suspect that patients who suffer persistent pain without evident cause are expressing some underlying psychopathology or using their sick roles for secondary gain. The usual "pain clinic" approach to such patients is to withdraw all analgesics, protect the patient from further invasive diagnostic and therapeutic interventions, establish rapport and trust, and retrain the patients to live with themselves and others in a more functional way. Most physicians regard analgesic drugs as ineffectual for such problems and possibly counterproductive; typically, referral clinics assume that the patient has already tried and failed all conventional therapies.

There is strong evidence that opioids are effective in relieving many chronic idiopathic pain even when all other treatments have failed. According to Taub (1982) patients with chronic intractable pain can be effectively treated with relatively low daily doses of opioids for prolonged periods up to 6 years with few management problems, in most cases without need to increase dose. He reported an incidence of inappropriate opioid usage of only 4.2% of 313 patients. Similar results with patients having a wide variety of chronic pain states not relieved by other methods, have been reported by Tennant and Uelman (1983) and France et al., (1984) reported similar results with patients having a wide variety of chronic pain states. In the most completely documented of these studies, Portenoy and Foley (1986) found that opioids provided partial or complete pain relief of chronic intractable pains in about 60% of the patients studied for up to 4 years. Half of their patients used the opioids for pain control for between 4 and 7 years on a daily basis and the daily dose used remained stable over time in nearly all cases.

There are other reports that chronic pain patients gain clear relief from short term opioid administration (Price et al., 1986; Gourlay et al., 1986a). It is thought-provoking to see that the effective plasma opioid concentration for relief of postoperative pain is exactly the same as for patients with chronic pain of nociceptive origin (Gourlay et al., 1986a). So far, there is only one voice of dissent in the research literature. In striking contrast to these and other reports, Arner and Meyerson (1989) claim that opioids are ineffective in similar patients with chronic idiopathic pains . Nonetheless, the weight of the evidence favors the efficacy of opioids in chronic pain. To understand the issue properly, we will need to determine those types of chronic pain for which opioids are ineffectual. They do not alleviate neuropathic pain states, for example. However, they modulate pain at the level of the CNS and therefore should work whenever there is a nociceptive component in the painful condition.

Unfortunately, we note a strong, decisive rejection of opioid therapy for chronic pain patients among many pain specialists. This is difficult to understand in light of the evidence that exists:

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these drugs can be beneficial for the right patients under the right circumstances. Perhaps physicians involved with chronic pain patients have inadequate understanding of opioids and therefore fear them inappropriately. The issues of most concern are tolerance, physical and psychological dependence and addiction.

Tolerance. Tolerance to opioids refers to a change in patient responsiveness to a drug such that more of the drug is required to produce a particular magnitude of effect. One of the most widely held misconceptions about opioids is that tolerance must develop. Even such experts as Nyswander and Dole (1986) state that "In summary, tolerance and physical dependence are inevitable consequences of repeated administration of narcotic drugs." Yet, reports of lack of tolerance development over years of daily opioid use in chronic pain patients continue to accumulate (Kanner and Foley 1981; Nyswander and Dole 1986). According to Twycross (1974), opioid tolerance is minimal in most cancer patients treated with oral doses of diamorphine for prolonged periods; dose increases, when required, usually follow increased pain due to tumor progression. Even when infused intrathecally for months for treatment of pain in cancer patients, morphine did not consistently induce tolerance (Yaksh and Onofrio 1987). Gourlay et al., (l986b) found that many cancer pain patients treated with daily doses of methadone or morphine in carefully tailored dosage regimens did not require dose escalation over time. Furthermore, the minimum effective plasma concentration of meperidine remained stable over prolonged periods (e.g., 12 months) of drug administration in chronic pain patients (Glynn and Mather 1982). Finally, in all of the studies of opioid treatment of chronic non-neoplastic pain described above pain relief was maintained with opioids without need for dosage escalation for years in the large majority of nearly 400 patients.

Physical Dependence. The clinical importance of "inevitable" physical dependence is often exaggerated. When tolerance does develop, physical dependence usually accompanies it. However, there are many reports of apparent lack of physical dependence in patients using opioids for pain control for years (Nyswander and Dole 1986; Twycross 1974; Glynn and Mather 1982). Physical dependence is not inevitable and it presents an easily controlled clinical situation when it does occur. As long as opioids are administered in appropriate doses and times, physical dependence is essentially transparent. Physical dependence becomes a clinical issue at the end of a treatment period and is readily managed in the large majority of patients by appropriate tapering of opioid intake.

Addiction. Investigators who have studied opioid treatment of chronic idiopathic and cancer pain agree that *de novo* addiction is very rare (Foley 1986). However, inappropriate use of opioids does occur. Drug abuse including alcoholism is a significant problem among patients with chronic pain. Maruta et al., (1979) reported that 41% of a chronic pain clinic population misused medication to a degree sufficient to warrant diagnosis as drug abusers. This is not surprising since depression occurs frequently

in this population and there is a correlation between rates of depression and drug abuse.

The abuse of medications is a serious problem among chronic pain patients seen at major referral clinics largely because well intentioned physicians feel that they must do something for the patient and can envision no other alternatives than a trial on medication. For some patients, medication prescription validates an otherwise questionable claim to invalid status to family or employer. When the drugs are ineffectual, patients self administer increasingly greater doses. This, in turn, causes side effects including affect disturbance; these symptoms of toxicity are then addressed with further prescriptions by physicians unaware of the patient's circumstances. Thus, to a large extent, the problems of drug abuse among chronic pain patients can be attributed to misprescription of psychoactive medications on the part of physicians. In their zeal to limit this problem, pain specialists have tended to obscure appropriate indications for opioids.

CANCER PAIN

Many recommend that cancer pain be treated with an "analgesic ladder" approach (WHO 1986). The notion that cancer pain is unidimensional is implicit in this strategy; the main goal is to decrease pain intensity and that can be achieved by using increasingly "stronger" analgesics. This approach is effective in about 70% of cancer pain patients. What about the remainder? Many cancer patients suffer with neuropathic pain along with their nociceptive pain. A recent report by Tanelian and Cousins (1989) illustrates the situation clearly for a patient with nociceptive and neuropathic pain associated with Pancoast tumor. In this case the patient's pain could only be made bearable by a combination carbamazepine and hydromorphone; either drug alone is clearly inadequate in patients having such a combination of chronic pains. Optimal treatment of cancer pain requires appreciation of the fact that many patients have a complex mixture of different types of chronic pain that may respond differentially to conventional and atypical analgesic drugs.

CONCLUSIONS AND RECOMENDATIONS

Chronic pain is not one thing; the term encompasses three classes of persisting pain problems. Moreover, patients sometimes suffer with more than one cause of pain, each of which may require different medication. Pharmacologic interventions can be of great value for chronic pain patients when the underlying problems are accurately diagnosed and the choice of medications is based on the type(s) of chronic pain. Sweeping policy statements that conventional analgesic medications are ineffectual or contraindicated when pain is chronic represent gross oversimplification and are counterproductive for all but perhaps the highly selected patient populations of certain major referral clinics.

The potential value of opioids for some chronic pain problems merits further exploration even though it is controversial because of the general apprehension that accompanies these drugs in our society. We propose on the basis of compelling literature that academic pain clinics undertake controlled trials of opioids with selected chronic pain patients. Comparisons between opioid management and behavioral/rehabilitative therapies in patients with chronic idiopathic pain could be of great value.

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ACKNOWLEDGEMENTS

Supported in part by Grants DA 05513 from the National Institute on Drug Abuse and CA 38552 from the National Cancer Institute.

AUTHORS

Harlan F. Hill, Ph.D., and C. Richard Chapman, Ph.D., Division of Clinical Research, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA, 98104.

Arthritic Inflammation in Rats as a Model of Chronic Pain: Role of Opioid Systems

Albert Herz, Mark J. Millan and Christoph Stein

Chronic pain represents a state of great significance both for the individual and for society as a whole. The pathology of chronic pain is multifarious and the cause often unknown. The detection of opioid receptors and endogenous ligands of these receptors initiated a new era in pain research. Most research has focused on the modulation of acute pain. In contrast, the role of opioids in the response to chronic pain has been the subject of only a limited number of studies, largely due to the experimental and ethical problems involved in such investigations. Only recently has the role of opioids in chronic pain attracted high interest and some aspects of this work shall be discussed in the present paper which concentrates on two models of chronic pain, polyarthritis and monoarthritis induced by Freund's adjuvant in the rat.

Opioid Research over the last 15 years has revealed a highly complex picture of endogenous opioid systems: a considerable number of opioid peptides deriving from three opioid peptide precursors are presently known; there is also a multiplicity of opioid receptors, the targets of these peptides; mu, delta and kappa receptors are the most important types. In view of the differential affinity and selectivity of various endogenous opioid peptides for these receptor types it has been suggested that dynorphin and related peptides represent the endogenous ligands of the kappa receptors; most probably the enkephalins are related to the delta receptors; so fax, however, no endogenous ligand of the mu receptors has been detected - although ß-endoqhin exhibits a certain preference for this receptor type. The mu receptor type seems to play a particularly important role in the modulation of pain; thus, it is not surprising that most of the conventional opioids used in pain therapy have a high affinity for this receptor type (Höllt 1986; Millan 1986).

POLYARTHRITIS AS A MODEL OF CHRONIC PAIN

Inoculation of rats at the tail-base with Mycobacterium butyricum leads to a generalized disease state; swelling and inflammation of the limbs and tail is a prominent feature which is acccompanied by an increased sensitivity to noxious pressure applied thereto; this observation, together with decreased appetite, weight loss, disruption of circadian rhythms, hyperventilation and, possibly, scratching suggests the animals may be experiencing chronic pain (Colpaert et al., 1980; Colpaert 1987; Millan et al., 1988; Calvino et al., 1987).

CHANGES IN OPIOID PEPTIDES

The most impressive changes seen in rats suffering from chronic arthritic pain is the rise in dynorphin levels in the lumbal dorsal horn. This increase (of up to 250%) reaches its maximum about 3 weeks after inoculation and parallels the time course of inflammation and hyperalgesia. Within 10 weeks these parameters become largely normalized. In the brain significant increases in dynorphin content are found only in the thalamus; ß-endorphin increases in the anterior pituitary and in plasma pointing to accompanying stress; minor but not always significant increases in methionine-enkephalin content are found in the spinal dorsal horn (Millan et al., 1986, 1987).

These data pointing to a particular role of dynorphin (and other peptides such as α-neo-endorphin deriving from prodynorphin) in the chronic pain state are supplemented by the finding that the expression of the prodynorphin gene under these conditions is considerably enhanced in the spinal cord (Höllt et al., 1987; Iadarola et al., 1988) indicating an increased functional state of the dynorphin system. This view is supported by the observation that in chronic arthritic rats, naloxone induces a stronger increase in firing of dorsal horn neurones than in control rats. This is roost easily explained by an increased inhibitory tone mediated by dynorphin (Ménétry et al., 1988). In this context it should be mentioned, however, that the in vitro and in vivo release of met-enkepahlin from the spinal cord of chronic pain rats was found to be decreased (Bourgoin et al., 1988; Cesselin et al., 1988; Przewlocki et al., 1986). (Dynorphin release has not been measured so far.) Possibly the enkephalins react differently than dynorphin.

OPIOID RECEPTORS

Some minor changes in opioid receptors have been found in the chronic arthritic state in rats: While the density of mu and delta opioid receptors did not change in brain and spinal cord, a small decrease in kappa opioid receptors was detected in the spinal cord (Millan et al., 1986). This finding of a sliqht down-regulation of this receptor type is in line with an increased dynorphinergic tone in the spinal cord in chronic arthritic rats (see above).

NOCICEPTIVE THRESHOLD

Nociceptive thresholds in chronic arthritic rats are affected differently: a pronounced hyperalgesia is observed when pressure is applied to the tailor the paw; in contrast, the nociceptive threshold is increased when radiant heat is used as a noxious stimulus. The supersensitivity to pressure is further enhanced by MR 2266, an opioid antagonist with benzomorphane structure exhibiting some selectivity for kappa opioid receptors (but not by application of naloxone which lacks selectivity for particular opioid receptor types). The effect of MR 2266 is stereospecific and not observed in the radiant heat test. These data suggest that an enhanced activity of dynorphin neurones in the spinal cord may, via kappa receptors, act to moderate the hypersensitivity of inflamed tissue to imposed noxious pressure: that is, dynorphin in this sense may combat polyarthritic pain (Millan et al., 1987). Further information, however, is required to confirm this hypothesis and its generality is not clear.

Of particular interest are the changes in the response of chronic arthritic rats to opioid agonists. Several papers show an increased antinociceptive response to mu and delta opioid receptor ligands (Millan et al., 1987; Neil et al., 1986; Kayser, 1988). Some discrepancies exist concerning kappa opioid receptor agonists. While Neil et al. (1986) report an increased duration of effect of U-50,488H, Millan et al. (1987) found a reduced effect of this kappa agonist in the paw pressure test. The mechanism of supersensitivity to morphine- like opioids which is not accompanied by an upregulation of mu receptors awaits clarification.

MONOARTHRITIS - UNILATERAL INFLAMMATION

A second model of prolonged pain has gained increasing importance in recent years: monoarthritis or, rather, unilateral inflammation induced by inoculation of Freund's adjuvant into the plantar surface of a single hind-paw in rats. From an ethical point of view, this model is advantageous as the intensity and generality of pain is much reduced in comparison to the polyarthritic model - as can also be deduced from measurments of stress hormones. In addition it has the advantage that the reaction of the inflamed paw and the non affected paw can be compared in the same animal. As will be shown below, this immediate comparison of both paws is an ideal model for studying peripheral antinociceptive effects of opioids manifest in inflamed tissue (Millan et al., 1988a; 1988b).

There are some important differences between the mono- and the polyarthritic model - although there are also close similarities between these. While in the case of polyarthritis there is a lag time of several days or even a week before the swelling develops, the swelling and inflammatory process in monoarthritis commences a few hours after inoculation and is quite strong after one day; however, also in this model the pathological processes persist for several weeks arid in sane cases the

inflammation spreads to the contralateral paw; the changes in opioid peptide levels resemble those seen in polyarthritis: an increase in dynorphin is the predominant finding; it is restricted to the dorsal horn of the affected side as long as the inflammation is restricted to one paw. No changes in opioid receptor density were found in the ipsi- as compared to the contralateral dorsal horn. Concerning nociceptive thresholds, similar changes were found as in the polyarthritic model: a decrease in threshold in the paw pressure test on the inflamed side which was further decreased by naloxone and MR 2266, indicating that endogenous opioids (in particular dynorphin) tend to counteract the inflamnation-induced hyperalgesia (Millan et al., 1988a; 1988b).

PERIPHERAL SITES OF OPIOID ANTINOCICEPTIVE ACTION

Several papers published in the last couple of years have claimed peripheral sites for the antinociceptive actions of opioids, although these reports are controversial and in some cases it is not even clear whether they are dealing with effects mediated by opioid receptors. The model of unilateral inflammation offers the opportunity to study possible peripheral antinociceptive/opioid effects (Stein et al., 1988a; 1988b; 1988c; 1989; Joris et al., 1987).

The key-finding indicating a peripheral site of opioid action in inflamed tissue was the observation that a low dose of morphine (2 mg/kg) greatly increased paw pressure thresholds in the inflamed paw, but did not change this threshold on the noninflaned contralateral side. Similar results were obtained with the selective kappa agonist U-50,488 (10 mg/kg) and further experiments showed that intraplantar injection of naloxone - but not its inactive (+)isomer - antagonized the effect of the systemically applied agonists (Stein et al., 1988b). In subsequent experiments, receptor selective opiods were applied intraplantarly. Low doses of DAGO (a mu receptor specific agonist), DPDPE (a delta receptor selective agonist) and U-50,488H (a kappa receptor selective agonist) exhibited antinociceptive effects in the paw- pressure test when injected into the inflamed paw, but not when injected into the non-inflamed paw. These effects were antagonized by the respective receptor-specific antagonists CTAP, ICI 174,864 and binaltorphimine (Stein et al., 1989). These findings suggest that opioids can produce antinociceptive effects by a local opioid receptor-specific mechanism in inflamed tissue. Several questions, however, remain to be clarified: which are the endogenous ligands for these receptors, what stimuli call them into play and what is the anatomical source of these endcgenous opioids. Studies addressing these issues are going on in our laboratory.

In summary, this chapter has reviewed some studies examining animal models of chronic pain. So far, the most widely used model is the polyarthritic rat. More recently, unilateral hindlimb inflammation has begun to replace the former, mainly for ethical and practical reasons. Significant findings

concerning alterations in endogenous opioid systems - in both models - are: Increased levels of dynorphin in the spinal cord and evidence for its role to counteract inflamnation-induced hyperalgesia. Exogenous opioids exhibit enhanced antinociceptive activity in the inflamed tissue which is apparently due to a recruitment of local opioid receptors therein. Future studies will focus upon the mechanisms of activation of these peripheral receptors and their endogenous ligands in prolonged inflammatory pain.

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AUTHORS

Albert Herz, Mark J. Millan and Christoph Stein
Department of Neuropharmacology
Max-Planck-Institut für Psychiatrie
Am Klopferspitz 18a
D-8033 Planegg-Martinsried, FRG.

Supported by Deutsche Forschungsgemeinschaft, Bonn.

Use of the Formalin Test in Evaluating Analgesics

A. Cowan, F. Porreca and H. Wheeler

INTRODUCTION

The choice of dilute formalin as a noxious stimulus in analgesic research is a relatively new development, the rat paw formalin test having evolved in only the past decade (Oubuisson and Dennis 1977; Abbott 1988; Bustamante et al., 1989). Formalin provides a continuous (tonic) background of pain that may be neurochemically and neurophysiologically different from the transient (phasic) pain associated with conventional hot plate and tail flick tests (Dennis et al., 1980; Abbott et al., 1982; Dennis and Melzack 1983). The corresponding test with mice has been developed by Hunskaar and his colleagues (Hunskaar et al., 1985; Hunskaar and Hole 1987) and others (e.g. Murray et al., 1988) while Alreja et al. (1984) have described a version of the test with rhesus monkeys. By studying tonic pain, the hope is that results from the animal laboratory will have a more direct bearing on drug therapy used in the persistent types of pain encountered clinically.

In our experience, the rat paw formalin test is proving to be an acceptable model (both ethically and pharmacologically) of steady prolonged pain. There is still a need to compare (in a comprehensive manner) key experimental opioids and clinically used analgesics against formalin. In the present work, morphine has been run in the model and compared with (a) PO 117302, a selective kappa agonist (Leighton et al., 1987; Clark et al., 1988) and (b) four narcotic antagonist analgesics that are used clinically - buprenorphine, butorphanol, nalbuphine and pentazocine.

MATERIALS AND METHODS

Animals. Male Sprague Dawley albino rats (70-90 g; Zivic-Miller) were used. They were acclimated to individual Plexiglas observation chambers (L, 21 cm; W, 25 cm; H, 30 cm) for at least 1 hr before testing.

Behavioral testing. After the acclimation period, each rat was injected s.c. into the dorsal surface of the right hind paw with

50 µl of 5% formalin or 50 µl of saline. Preliminary experiments have revealed two spontaneous behaviors indicative of pain: (a) flinching/shaking of the paw and/or hindquarters, sometimes observed as a rippling motion across the back, and (b) licking/biting of the injected paw. These behaviors were monitored between 0-10 min (early/acute phase) and 20-35 min (late/tonic phase) following the injection of formalin or saline.

Assessment of antinociceptive activity. The potencies of morphine sulfate (Mallinckrodt) and PD 117302.HCl, that is, (±,I-trans-N-methyl-N[2-(l-pyrrolidinyl)-cyclohexyl]benzo[b]-thiophene-4-acetamide (Parke-Davis) were assessed in both early and late phases of the formalin model. Buprenorphine HCl (NIDA), butorphanol tartrate (Bristol Labs), nalbuphine HCl (Endo Labs) and pentazocine HCl (Sigma) were only evaluated against the late phase. Doses are expressed in terms of the free base.

Test compounds were injected s.c. Pretreatment times were chosen so that peak antinociceptive activity coincided with the observation period. For early phase behaviors, morphine and PD 117302 were given 20 min and 30 min, respectively, before the formalin. For late phase behaviors, PD 117302 was injected 10 min before the formalin; the other compounds were injected immediately before the formalin.

Separate groups of rats (n = 5-10 per dose) were used to generate dose-response curves in each phase of the response and for each agent. Results are expressed as mean % antagonism of formalin-induced flinching (or licking) ± S.E. and are calculated for individual drug-treated, formalin-injected rats as follows:

$$\frac{\text{(Mean F response-mean saline response)-Individual response}}{\text{(Mean F response-mean saline response)}} \times 100$$

where "mean F (formalin) response" is the mean behavioral score obtained in concurrently run vehicle-treated, formalin-injected rats, and "mean saline response" is the pooled behavioral score for rats injected with 50 µl saline into the hind paw.

Antinociceptive potency is expressed as the A_{50} and 95% confidence limits and was obtained by linear regression from the % antagonism of formalin-induced responses.

RESULTS AND DISCUSSION

The recording of flinching and licking, at 5 min intervals for 90 min post-formalin, clearly demonstrated a biphasic response for both behaviors (figure 1). An immediate (acute/phasic) effect (0-7 min) was followed by a prolonged (tonic) response which, for flinching, was maximal between 20 and 50 min and persisted for up to 80 min. Late phase licking was of shorter duration (18-38 min). We have found licking to be much more variable than flinching. In our opinion, flinching is the more reliable measure in the rat for evaluating different types of analgesic.

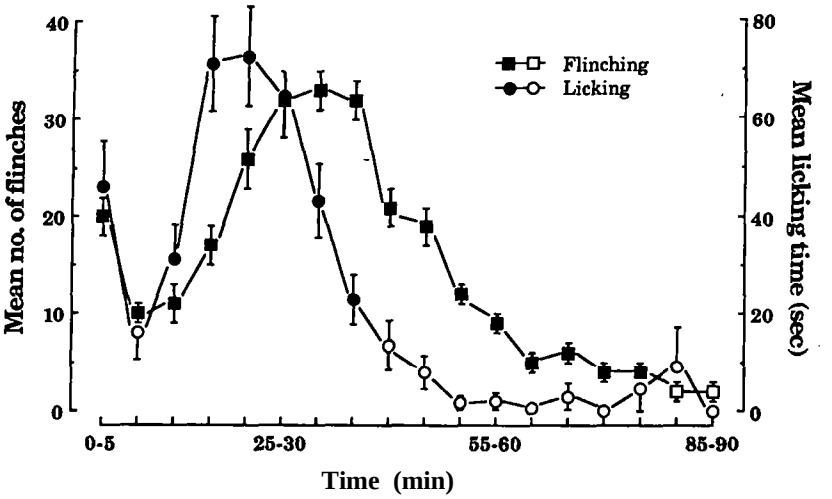

FIGURE I. Time course of flinching and paw licking in rats (n = 17) after s. c. injection of formalin into the right hind paw at T= 0. Each point is the mean behavioral sCOre per 5 min ± S.E. Closed symbols represent values which are significantly different from controls (injected with saline into the hind paw); P<O.05 (Student's t test).

Despite the preceding cautionary remark, morphine was exceptionally consistent as an antinociceptive agent in the rat paw formalin model. Thus, A_{50} values for morphine against early (1.1 mg/kg, 0.9-1.4) and late (0.6 mg/kg, 0.5-0.7) flinching and early (1.8 mg/kg, 1.1-3.7) and late (0.7 mg/kg, 0.5-0.8) licking were comparable.

The situation was very different with PD 117302. This kappa agonist was 27 times more potent against late flinching (0.2 mg/kg, 0.09-0.3) than against early flinching (5.2 mg/kg, *3.5-8.9)* (figure 2). In contrast, PD 117302 was 5.3 times more potent against early phase licking (0.06 mg/kg, 0.02-0.11) than against late phase licking (0.4 mg/kg, 0.3-0.6). However, as with morphine, there was a relatively poor correlation between the dose of PD 117302 and antagonism of formalin-induced licking during the early phase. We therefore believe that, based on the flinching results, the tonic pain state has a greater influence on kappa, as opposed to mu, systems.

Reproducible dose-response lines were obtained with the narcotic antagonist analgesics assayed in the formalin model. The rank order of potency for attenuating flinching after s.c. administration was buprenorphine>nalbuphine>butorphanol>morphine>pentazocine (table 1). Four out of the five agents were (like PD 117302) fully efficacious against flinching, the exception being nalbuphine (figure 3). Efficacy was also lower for this compound after i.c.v. and intrathecal administration (unpublished results).

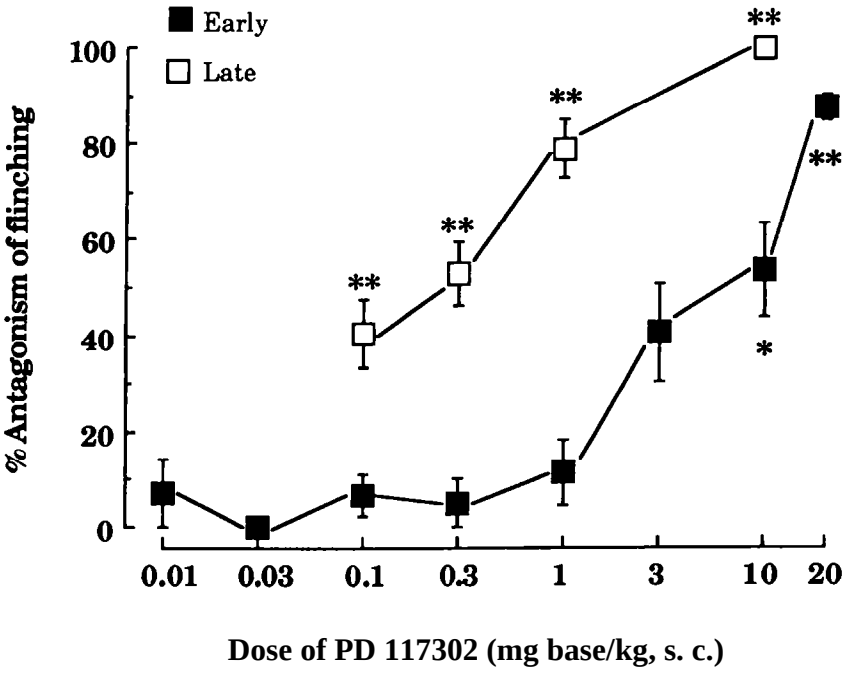

*FIGURE 2. Dose-response lines for PD 117302 for antagonism of early phase (0-10 min) and late phase (20-35 min) flinching induced by Formalin in rats. Each point is the mean % antagonism of formalin control ± S.E. Significant difference from formalin + water was determined with ANOVA followed by Dunnett's test (*P<0.05; **P<O.01).*

TABLE 1. Effect of Test Agents on Late Phase flinching in the Rat Paw Formalin Test.

	A_{50} (mg/kg, s.c.)
Buprenorphine	0.03 (0.02-0.04)
Nalbuphine	0.08 (0.04-0.35)
Butorphanol	0.1 (0.02-0.4)
Morphine	0.6 (0.5-0.7)
Pentazocine	0.9 (0.7-1.2)

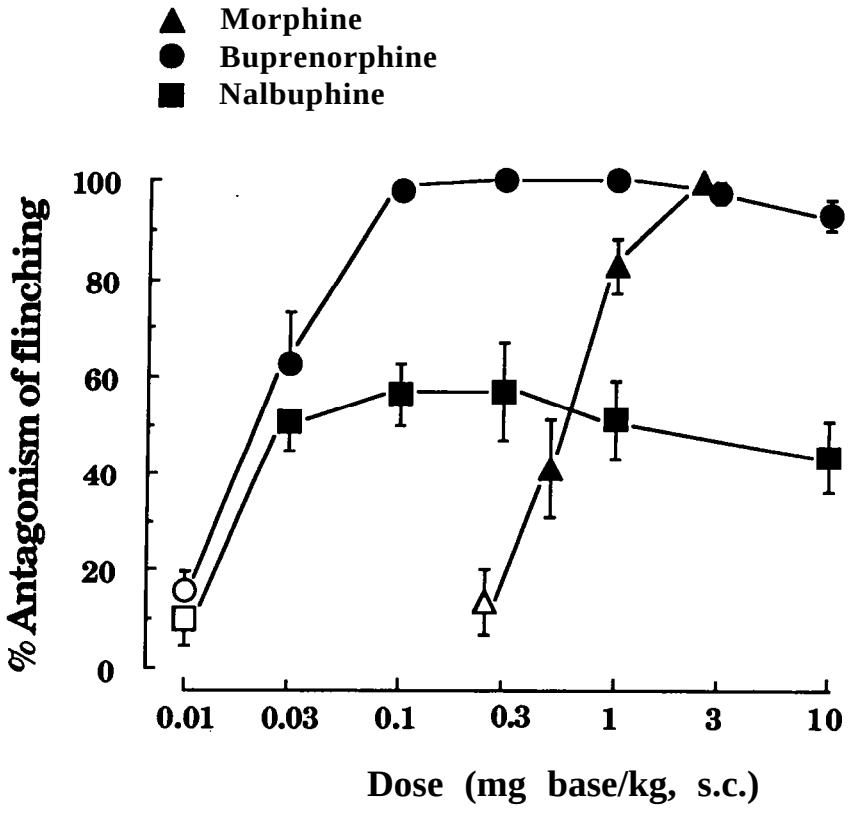

FIGURE 3. Dose-response lines for morphine buprenorphine and nalbuphine for antagonism of late phase flinching induced by formalin in rats.

In conclusion, formalin evokes a robust, spontaneous response (flinching) in rats - ideal for testing analgesics. Formalin may be a noxious stimulus of choice in assessing the efficacy and antinociceptive potency of kappa agonists/mixed agonist-antagonists. The rat paw formalin test as described may be utilized in a meaningful way to investigate both phasic and tonic aspects of pain. Finally, receptor-selective agents (as exemplified by morphine and PD 117302) show differential activities in the model depending upon the phase of pain examined and the nature of the endpoint under study.

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ACKNOWLEDGEMENT

This work was supported by Grant DA 03945 from NIDA.

AUTHORS

Alan Cowan, PhD and Helen Wheeler, BSc
Department of Pharmacology
Temple University School of Medicine
Philadelphia
PA 19140

Frank Porreca, PhD
Department of Pharmacology
University of Arizona Health Sciences Center
Tucson
AZ 85724

Nicotine Abstinence Effects

John R. Hughes

The valid effects of tobacco abstinence include slowing of the EEG. increased REM density with subsequent sleep awakening, decreased heart rate, thyroid functioning, and performance and increased caloric intake, sweet taste, weight, anger, anxiety, difficulty concentrating, restlessness, and impatience.

Tobacco withdrawal effects possess within-and between-subject reliability, are prevalent (50 - 80%) and can be of clinical magnitude. Tobacco withdrawal peaks in 1 - 2 weeks and declines to pre-cessation levels by 4 weeks with no evidence of protracted withdrawal except for hunger and craving. Several lines of evidence indicate tobacco withdrawal is due in large part to nicotine deprivation.

Tobacco withdrawal is similar to classical drug withdrawal syndromes in that animal models are available, it is associated with tolerance, has some evidence of symptom stages, 'and is reduced by gradual reduction. Tobacco withdrawal is dissimilar to classical drug withdrawal syndromes in that antagonist precipitated, protracted and conditioned withdrawal have not been demonstrated. The role of tobacco withdrawal in smoking relapse is unclear.

AFFILIATION

Human Behavioral Pharmacology Laboratory, Departments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401

Disruption of Schedule-Controlled Behavior During Abstinence from Phencyclidine and Tetrahydrocannabinol

Robert L. Balster

The repeated administration of many drugs of abuse results in the production of dependence as evidenced by an abstinence syndrome upon discontinued exposure. Animal models that have been developed for studying drug dependence have traditionally relied on weight loss and/or directly observable signs of drug withdrawal. Using this approach it has been difficult to establish animal models for dependence on drugs such as cocaine and amphetamine, phencyclidine (PCP), tetrahydrocannabinol (THC), caffeine and nicotine. This presentation reviews some studies which have used the disruption of schedule-controlled responding during drug withdrawal in rats and monkeys as a more quantitative and subtle measure of drug abstinence effects, with a particular focus on our research with PCP and THC. The pioneering research with morphine by Thompson and Schuster (1964) and Holtzman and Villarreal (1973) in rhesus monkeys provides the background for this approach.

Based upon evidence we obtained for an observable PCP withdrawal syndrome in rhesus monkeys self-administering very large doses of intravenous PCP (Balster and Woolverton, 1980), subsequent research found that disruption of food-maintained fixed-ratio performance in monkeys and rats occurred 12-16 hours after discontinuation of intravenous infusion of doses of PCP which had little, if any, effects on behavior during treatment (Slifer et al., 1984; Beardsley and Balster, 1987). These behavioral effects occurred without clear observable signs of withdrawal, were immediately reversed by re-administration of PCP, and showed recovery after a few days. Cross-dependence between PCP and ketamine was also demonstrated using this model. We were also able to characterize a dependence upon THC in rhesus monkeys using this approach; however, in this case, the onset of behavioral disruption was delayed by a day or more, consistent with the slower elimination of THC than PCP (Beardsley et al., 1986). THC abstinence produced disruption of behavior could also be reversed by re-administration of THC.

In a recent study by Woolverton and Kleven (1988) using this same general approach, continuous infusion of cocaine was able to produce disruption of operant behavior during withdrawal; however, the relative dose and duration of treatment required was quite extensive. A recent study by Carroll et al. (1988) illustrates behavioral studies of caffeine dependence. This study also illustrates the disruption of behavior during abstinence from a drug which has been self-administered, thus combining studies of drug reinforcement and dependence.

The use of alterations of learned behavior in animals during drug withdrawal provides a very useful tool to study dependence. With the increased ability to detect subtle drug abstinence effects, it may became necessary to re-evaluate traditional definitions of physical dependence. It has been proposed to use the term behavioral dependence on drugs to describe the condition where subtle behavioral effects during abstinence are the primary indices of withdrawal (Balster, 1985). It may be that for- some drugs and/or under some conditions it may be useful to make distinctions between physical and behavioral dependence.

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ACKNOWLEDGEMENTS

The PCP and THC research was supported by National Institute on Drug Abuse Research Grants DA-01442 and DA-00490. The scientific contributions of Drs. Patrick Beardsley, Louis Harris, Barbara Slifer, and William Woolverton to this research were substantial and are gratefully acknowledged.

AFFILIATION

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

Anxiogenic Effects of Drug Withdrawal

M. W. Emmett-Oglesby

Drug withdrawal syndromes consist of signs (objectively observable) and symptoms (subjectively experienced). Traditionally, classes of drug withdrawal giving rise to signs are said to cause physical dependence, whereas classes of drug withdrawal giving rise to symptoms are said to cause psychological dependence. However, both phenomena are expressions of physiological reactions to drug withdrawal, and rather than assigning drug withdrawal to one of these two classes, it is probably more appropriate to conceptualize all drugs of dependence as producing withdrawal syndromes that involve mixtures of both signs and symptoms. Subjective events cannot be studied in animals, so it is not surprising that our knowledge of symptoms of drug withdrawal is less complete than our knowledge of signs of drug withdrawal. Nonetheless, withdrawal from dependence on drugs such as benzodiazepines and cocaine produce symptoms that appear to be critical in maintaining drug dependence, and animal models may be useful in helping to understand these problems. One symptom of withdrawal shared across many drugs of dependence is anxiety, and it should be possible to use animal models of anxiety to investigate this aspect of drug withdrawal. This paper reviews the results with one such model, discrimination of the anxiogenic drug, pentylenetetrazole (PTZ), by rats. In this technique, rats are trained to discriminate PTZ using a food-reinforced choice-task. Prior to chronic treatment with a benzodiazepine, the benzodiazepine antagonist, flumazenil, does not substitute for PTZ. However, following a week of diazepam administration, 60 mg/kg/day, flumazenil substitutes for PTZ in a dose-related manner (e.g., Emmett-Oglesby et al. 1987, 1988). In experiments with ethanol, immediately following four days of ethanol, rats selected the saline lever; by 12 to 18 hours after the last dose of ethanol, they selected the PTZ lever, and by 48 to 72 hours after terminating ethanol, they again selected the saline lever. The amount of PTZ-lever selection was proportional to the duration of ethanol administration and was a direct function of the daily dose of eth-

anol administered (Lal et al. 1988). In experiments with chronic cocaine -administration, after terminating a two week course of 60 mg/kg/day of cocaine administration, rats initially selected the saline lever, but by 48 hour selected primarily the PTZ lever, and at two-weeks after stopping cocaine, they again selected the saline lever (Wood at al. 1989). These data are consistent with the hypothesis that a PTZ-like stimulus arises during withdrawal from a variety of drugs. Because drugs such as beta-carbolines, that substitute for PTZ are anxiogenic in humans, these data further suggest that the PTZ discrimination has utility for investigating an aspect of drug withdrawal that may be related to the human problem of anxiety. Such a model should be useful for exploring treatments that alleviate this problem, as well as for understanding the neurobiologic bases of this aspect of withdrawal.

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ACKNOWLEDGEMENTS: Supported by NIDA grant DA RO1-3521 and NIAAA grant AA RO1-6890

Affiliation: Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690

Caffeine Abstinence Effects in Humans

Roland R. Griffiths

Although caffeine is the most widely used behaviorally active drug in the world, caffeine physical dependence has been only moderately well characterized in humans and is not widely recognized by the lay population or by health-care professionals. For example, the most recent version of the influential diagnostic manual of the American Psychiatric Association (DSM-III-R) does not acknowledge the existence of clinically significant caffeine physical dependence. In fact, physical dependence is among the most clearly demonstrated adverse effects of habitual caffeine use. Such dependence is manifested by biochemical, physiological, behavioral, and subjective disturbances which occur upon termination of chronic drug administration. Thirty-three published reports--including clinical observations, case studies, survey studies and experimental manipulations, which provide information about the signs, symptoms and time course of the caffeine withdrawal syndrome--have been reviewed recently (Griffiths and Woodson, 1988). Headache and fatigue are the most prominent caffeine withdrawal symptoms, with a wide variety of other signs and symptoms having been reported at lower frequency (e.g., anxiety, rhinorrhea, irritability, impaired psychomotor performance, nausea/vomiting, yawning, insomnia, confusion, diaphoresis, muscle pains/stiffness, coffee craving). When signs and/or symptoms of caffeine withdrawal occur, the severity can vary from mild to extreme. At its worst, caffeine withdrawal has been repeatedly documented to be incompatible with normal functioning and sometimes totally incapacitating. The withdrawal

syndrome follows an orderly time-course, with onset occurring at 12 to 24 hours, peak occurring at 20 to 48 hours, and duration most often being described as being about 1 week. The pharmacological specificity of caffeine withdrawal has been established by the observations that: 1) severity is an increasing function of maintenance dose; 2) withdrawal occurs after administration of caffeine in capsules as well as in beverages; 3) caffeine withdrawal symptoms are suppressed by administration of caffeine in capsules, tablets, or beverages; 4) magnitude of suppression is an increasing function of dose; and 5) caffeine is more effective at suppressing withdrawal than a variety of other drugs. The proportion of heavy caffeine users ($\geq$500 mg/day) who will experience symptoms after caffeine abstinence is unclear; however, the proportion has been reported to be greater than 80% in a study using a relatively unselected subject population. There has been wide variability, perhaps reflecting stable individual differences, in incidence, severity and duration of withdrawal symptoms. Although the minimum conditions necessary to induce physical dependence are also unclear, there is evidence for withdrawal symptoms occurring after termination of caffeine after short-term exposure to high doses ($\geq$600 mg/day for 6 to 15 days) or after long-term exposure to relatively low doses (100 mg/day). Several studies suggest that physical dependence may substantially potentiate the reinforcing effects of caffeine.

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(Supported by U.S.P.H.S. Grant R01 DA03890)

AUTHOR

Roland R. Griffiths, Ph.D.
Department of Psychiatry and Behavioral Sciences
Department of Neuroscience
The Johns Hopkins University School of Medicine
720 Rutland Avenue, Baltimore, MD 21205, U.S.A.

Behavioral Assessment of Subtle Drug Abstinence Effects: Overview and Discussion

Joseph V. Brady

The practices associated with the assessment of drug-related abstinence effects have traditionally relied upon physiochemical characterization and physiological activity in relationship to known standards of pharmacological equivalence. Such abstinence effects, popularly referred to as withdrawal, have long been the defining characteristics of drug abuse disorders, constituting evidence of so-called physical dependence. This concept has conventionally been applied rather narrowly to describe only conditions involving continuously repeated drug administration leading to a syndrome of biochemical and physiological effects with a characteristic time course corresponding to active drug elimination following cessation of drug intake and reversible by drug re-administration. The increasingly prominent role of more refined behavioral methodologies however, has extended the range of such evaluations and provided a more comprehensive basis for analysis of a drug's abstinence effects. The resulting advances in knowledge about drug actions, and particularly in research technology, as the presentations in this symposium clearly document, have made possible a more operational approach to pharmacological assessment of abused drugs and have called attention to the need for reappraisal of traditional concepts and definitions in the field.

The fact, for example, that a range of the most commonly used and abused drugs including nicotine, caffeine, and even marijuana and cocaine, under an equally broad range of self-administration conditions were not thought to be associated with the more obvious biochemical and physiological abstinence effects has long diverted attention from their dependence potential and delayed full appreciation of their abuse liability. It is now abundantly clear however, largely as a result of the research in which the participants in this

symposium have been pioneers, that behavioral assessment methodologies can provide a framework for the analysis of subtle drug abstinence effects without constraining such evaluations by limiting definitions to conditions that produce the more traditional physical dependence withdrawal syndrome. Indeed, it is now well documented that the development of more sensitive behavioral assessment procedures can reveal abstinence effects under conditions where the typical biochemical and physiological syndromes are either not observed or do not covary with the measured behavioral changes.

The most obvious contribution to be recognized in this current series of presentations is of course methodological. John Hughes has called attention to some important distinctions to be made both from topographic and functional perspectives in confronting the vast array of effects that follow drug withdrawal. Moreover, the presentation by Roland Griffiths impressively documents the generalizeability of these categorical changes to a caffeine withdrawal data base that extends the range of John Hughes' nicotine abstinence effects.

A second major strength revealed by the methodological advances detailed in these presentations resides not only in their increased sensitivity to abstinence induced behavioral changes, but as well in the greater degree of quantitative objectivity that they provide when compared to more traditional procedures that rely solely on observational techniques. Bob Balster's analysis of THC and PCP abstinence effects based upon schedule-controlled response rate and pattern changes for example, documents the quantitative nature of such abstinence effects. Moreover, this approach is advantaged by a methodology that reveals the graded expression of such effects by providing measurement of a continuous variable unlike the nominal variables conventionally used in such abstinence assessments (e.g. vocalization, piloerection, convulsions, etc.).

But these presentations represent more than a refinement of existing behavioral methodologies and their rigorous application to the assessment of subtle drug abstinence effects. They call attention as well to the novel initiatives that drug abuse research has spawned in extending what used to be called state dependent learning and is now familiarly known as drug discrimination. It has become increasingly clear, for example, that the assessment of so called subjective effects or symptoms (as contrasted with objectively measured signs) need not be the exclusive domain of our human subject research colleagues. The application of

this methodology to the laboratory animal evaluation of pharmacological agents has added a new dimension to the assessment of subtle drug abstinence effects.

Michael Emmett-Oglesby's work for example, has demonstrated convincingly that animals can be trained in much the same way that speaking humans are trained, to emit a discriminated operant in reporting the symptomatic effects of precipitated withdrawal. Moreover, while this approach requires no gratuitous assumptions about what aspect of the abstinence syndrome is being discriminated, it does hold promise of providing somewhat more specific information about the nature of the interoceptive changes involved. In this regard of course, some caution must be exercised in adapting models suggested by such clinical vernacular as "anxiety". While the realities to which such referents call attention can not be denied, their specification in "pop psychology,, terms obviously leaves much to be desired. It is indeed a sad commentary on our beloved "psych" desciplines that they remain unique among the fields aspiring to scientific status in embracing terminology taken from common usage as though it were a technical language. Mathematicians have their symbols and equations, chemists their structures and formulas, and even physicists have their quarks. Only the disciplines indentified by the "psych" prefix persist in their recourse to street corner terminology with all its excess meaning, as a basis for technical reference.

Polemics aside, perhaps the most significant implication of the subtle abstinence effects revealed by the assessment procedures described in these presentations relates to still unsettled issues regarding the extent to which such residuals of drug use contribute to the maintenance of abuse performances and to relapse in those numerous cases where our most effective treatment interventions prove evanescent. The broad environmental perspective we have all come to appreciate in confronting substance use and abuse problems emphasizes not only the complexity of the etiologic, maintaining, and relapse factors that characterize such disorders but as well the web of social myths that surround this domain. These myths and expectations function in curious ways to exacerbate the problem by perpetuating notions and explanations that endow terms like "addiction" "loss of control" and particularly "abstinence avoidance', with undeserved explanatory powers. One important advantage of the research approaches described is their generally operational orientation, which, though certainly not culture-free in any literal sense, does strive for definitional precision in clarifying the variables that

characterize the abstinence syndrome as well as the range of associated biomedical and behavioral antecedents and consequences.

Of particular significance in this regard of course is the convincing demonstration that these subtle abstinence effects can now be detected, recorded, and quantitatively measured, and that their conditions of occurrence can be specified with accuracy and precision. Under such circumstances, it should now be possible to provide more definitive answers to the abiding questions of how and to what extent these subtle behavioral changes following withdrawal from self-administrated drugs of abuse contribute to the persistence and reoccurrence of such disorders, as well as their interactive effects upon treatment and prevention. The operational form in which such questions must of course be put would seem to focus upon changes in the reinforcing function of a drug in relation to the nature and degree of such abstinence effects. The experimental designs required to answer such question may not be as straightforward as we would like, but they are certainly not beyond the technical advances in the field.

There are of course several other important implications of the subtle abstinence effects findings reported in these presentations that require at least brief mention. The generally accepted pharmacological lore about relationships between tolerance and dependence (i.e. abstinence) has not been the focus of this symposium, of course, but the data upon which these extensive analyses and discussions have been based would seem to hightlight the need for a closer look at the essential features of these presumably interacting processes. It is certainly clear from these presentations that the more obvious indicators of drug tolerance effects do not seem to be a necessary precursor condition for demonstrating the subtle abstinence effects described. And from a more systematic perspective, these remarkably detailed and sensitive evaluative analyses would seem to require that those of us who have found it so easy to distinguish between the abuse and dependence processes on the basis of operationally defined but somewhat over-simplified all-or-none criteria take another look at our self-evident solutions to the conceptual problems that continue to plague the field.

AUTHOR:

Joseph V. Brady
Johns Hopkins University School of Medicine
Baltimore, Maryland

Assessing the Reinforcing Properties of Drugs

Chris-Ellyn Johanson

Drug self-administration studies using humans as experimental subjects are few in number compared to those using animal subjects. Nevertheless, human drug self-administration research has made important and unique contributions to our understanding of the reinforcing properties of drugs relevant to drug abuse. Further, despite their low frequency, the goals pursued in these studies have been broad. This paper will review these various goals by describing selected examples of individual studies. The use of specific examples to describe research goals will also enable those unfamiliar with this type of human research to appreciate the range of conditions under which such studies can be conducted. For those interested in more extensive reviews, I recommend Griffiths et al (1980) and Henningfield et al (1986).

ESTABLISHING DRUGS AS REINFORCERS

For each new type of drug studied in humans, it is initially essential to determine whether the drug functions as a positive reinforcer before additional research questions can be pursued. In addition to determining whether responding is maintained by contingent drug delivery, such studies often evaluate the effects of a variety of environmental variables. The rationale of this approach is that if the drug is serving as a reinforcer, the responding it maintains should change when manipulations are made that have been shown to alter responding maintained by other events. In addition, if the reinforcing properties are related to the pharmacological actions of the drug, appropriate pharmacological manipulations should also be expected to modify responding. An additional test of whether a drug is functioning as a reinforcer is to compare results in human studies with those obtained with animals. In animal studies, it is more feasible to fully characterize the reinforcing properties of drugs. Thus, if under the more limited conditions used with human subjects results are comparable, the likelihood that these results are valid is increased. But likewise, by demonstrating comparability with the results obtained with humans, the validity of animal models is better established

Methods

In general, the types of procedures that have been used in animal studies have been modified for human self-administration studies. Modifications have been necessary to decrease any risk of toxicity and have included carefully selecting participants, using relatively low doses, limiting drug exposure within experimental sessions, and minimizing the length of the experiment.

Two types of dependent measures have been used to measure the reinforcing properties in most human drug self-administration studies. Henningfield and Goldberg (1983) used a free-operant procedure to evaluate i.v. nicotine in human cigarette smokers. Nicotine (1.5 mg) was available to subjects by responding on a lever under a fixed-ratio 10 schedule of drug delivery. The rate of injections self-administered during each 3-hr session was used as the measure of reinforcing properties. Subjects who had no history of drug abuse (except for nicotine cigarettes) initially self-administered few injections. Over time, however, rate of injections increased. As further evidence that nicotine was functioning as a reinforcer, these investigators showed that responding declined dramatically when saline was substituted for nicotine.

Choice measures have also been used in human drug self-administration studies. Johanson and Uhlenhuth (1980a) evaluated the reinforcing properties of 5 mg d-amphetamine administered orally in normal human volunteers without a history of drug abuse. In their procedure, there were 9 experimental sessions and subjects were not required to remain in the laboratory after ingesting the drug capsule. During the initial four sessions, subjects received drug and placebo twice on separate sessions. During the last 5 sessions, subjects were given a choice of which capsule they preferred and the measure of reinforcing properties was the number of times drug was selected. They found that amphetamine was chosen over placebo on 4 out of 5 sessions and considered this evidence that amphetamine was functioning as a reinforcer.

Effects of Behavioral Manipulations

In behavioral studies using other types of reinforcers, increasing the behavioral requirements can result in a decrease in the number of reinforcers obtained. Similar findings have also been obtained with drug reinforcers. Bigelow et al (1976) evaluated the reinforcing properties of sedatives in male subjects with a history of sedative abuse who resided on a research ward. A maximum of 20 doses was available during daily 7-hr sessions; the drug available on different days was either 30 mg pentobarbital or 10 mg diazepam. Subjects were required to ride an exercycle for 2 minutes to obtain a single token which could be exchanged for drug or money. When the cost per ingestion was 1 token, subjects took almost all available (20) doses of both drugs. However, as the requirement increased to 3, 5 or 10, the number of doses self-administered decreased. For instance, when the cost/dose was 3 tokens, approximately 5 doses of pentobarbital were self-administered whereas all 20 doses of diazepam were self-administered. When the cost increased to 10 tokens, self-administration of pentobarbital was totally suppressed whereas about half of the diazepam doses were still self-administered. The relative sensitivity of responding maintained by pentobarbital to response requirements has also been shown in animal studies (Goldberg et al. 1971).

In a similar study using the same experimental paradigm and doses of pentobarbital and diazepam, Griffiths et al 1976) showed that drug self-administration was also affected by the minimum interingestion interval between doses. When the interval was 0, the exercycle riding requirement was completed for approximately 75% of the available doses. However, as the interval increased, responding declined and this decline was more pronounced for pentobarbital than for diazepam

In summary, early drug self-administration studies clearly demonstrated that the paradigms used for humans were sensitive to behavioral manipulations that have been shown in other studies to modify maintained responding. Such sensitivity was

further evidence that the drugs were functioning as positive reinforcers and that responding maintained by drugs in an experimental setting with humans as subjects was predictable.

Effects of Pharmacological Manipulations

As a method of verifying that the response-maintaining characteristics of drugs are related to their other pharmacological properties, drug self-administration studies have evaluated the effects of manipulating dose, or magnitude of reinforcement. Relative to animal studies, it is somewhat difficult to generate a complete dose-response function with humans because of potential toxicity at the higher doses. Nevertheless, many studies have succeeded in evaluating a wide enough dose range to demonstrate that responding in humans can be influenced by the magnitude of drug reinforcement. Henningfield and Griffiths (1980), for instance, showed that if the dose of nicotine per puff was increased, rate of puffing (drug self-administration) decreased. This result is similar to the results obtained using animal subjects with a variety of drugs including i.v. nicotine under conditions (e.g., ratio schedules) where rate of responding influences drug intake (Johanson and Schuster 1975). That is, as dose is increased, rate of responding decreases. In contrast, with reinforcers such as food, rate of responding tends to increase with magnitude. The drug self-administration results have been interpreted as demonstrating that other properties of the drugs influence rate of responding non-specifically (Johanson 1978). Therefore, in animal studies, non-rate procedures such as drug choice paradigms have been used and it has been shown that relative choice increases with dose (Johanson and Schuster 1975). Using a similar choice paradigm with humans, Fischman and Rachlinski (1989) also showed that choice of cocaine increased as the dose available increased.

Comparison of Animal and Human Studies

The results described above not only indicate that the procedures that have been developed for human self-administration studies are sensitive to a variety of independent variables, but also underscore the similarity between the results obtained in human and animal studies. Another extremely convincing example of the comparability between human and animal studies can be seen in Figure 1. Several investigators have shown that local anesthetics, such as procaine, can maintain responding in rhesus monkeys (Johanson 1980). Because procaine is not abused by humans, the procaine results appeared to be an exception to the general finding that animals self-administer the same drugs that humans abuse (Johanson and Balster 1978). However, Fischman has shown that procaine is also a reinforcer in humans (Fischman 1989). In a choice paradigm, procaine was clearly selected over saline but when given a choice between 8 mg cocaine (i.v.) and different doses of procaine up to 96 mg, cocaine was overwhelmingly preferred. Likewise, in a study using rhesus monkeys, Johanson and Aigner (1981) also showed that procaine was preferred over saline but that when given a choice between cocaine and procaine, cocaine was preferred even when there was a 16-fold difference in dose.

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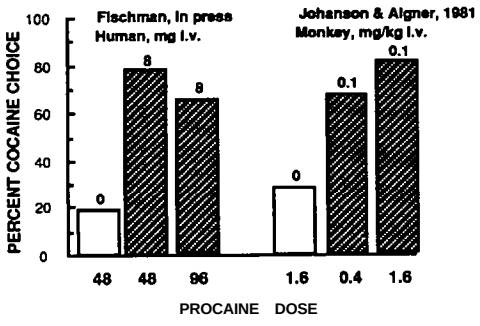

Figure 1: The number of choice trials during which cocaine (at the dose indicated above each bar) was selected over the dose of procaine shown below the bar. See text for details.

A second example of the similarity between the results obtained with animals and those obtained with humans can be seen in Figure 2 which shows the pattern of responding maintained by ethanol in both humans and rhesus monkeys.

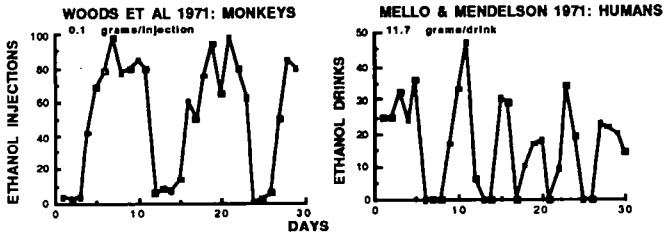

Figure 2: Pattern of ethanol self-administration in monkeys and humans. See text for details.

ASSESSING DEPENDENCE POTENTIAL

One important goal of drug self-administration studies is to evaluate the dependence potential of drugs. If human studies are to be useful in this regard, it is necessary to demonstrate that the results generated under laboratory conditions correctly predict which drugs would be abused. Thus, some studies have compared the reinforcing properties of drugs that are known to be abused by humans with other CNS drugs which are not drugs of abuse. For instance, Griffiths et al (1979) showed that responding was maintained by pentobarbital but in the same drug-experienced subjects, chlorpromazine was not a reinforcer. Likewise, Johanson and her colleagues using a choice paradigm showed that under the same conditions where responding was reliably maintained by &amphetamine. other psychomotor stimulants that are also abused, such as phenmetrazine, were also selected over placebo, whereas drugs such as fenfluramine and mazindol which are not abused by humans were not self-administered (Chait, et al. 1987. Johanson and Uhlenhuth 1980a, Johanson and Uhlenhuth 1982). Given these differential findings, it appears that results from human studies designed to assess dependence potential of new drugs are likely to yield valid predictions.

Drug self-administration studies can be difficult and time-consuming to conduct in humans. Traditionally, measures of subjective effects have been used to assess

dependence potential. One goal of human drug self-administration studies has been to evaluate the relationship between subjective effects and drug self-administration. For instance, de Wit et al (1986) compared the subjective effects of amphetamine in those subjects who preferred this drug over placebo in a choice paradigm with the subjective effects produced in subjects who did not prefer amphetamine, and found predictable differences between the subjective response of these two groups. Chait et al (1987) using a similar choice paradigm with normal volunteers evaluated the reinforcing effects of several pharmacologically related psychomotor stimulants and found that the profile of subjective effects was similar for those drugs that were self-administered compared to those that were not. However, Roache and Griffiths (in press) did not find a reliable relationship between measures of self-administration and drug liking with benzodiazepines in subjects who were previous sedative abusers residing on a research ward. Differences between subjective effects and level of drug self-administration have also been found in other studies (Johanson and de Wit 1989). Thus, while subjective effects may be useful predictors of dependence potential, the independent assessment of reinforcing properties in drug self-administration studies may add important information for reliably predicting the probability that humans will abuse a particular drug.

The assessment of the discriminative stimulus effects of drugs has been used in animal studies to indicate dependence potential. In a series of studies that evaluated both the reinforcing and discriminative stimulus effects of psychomotor stimulants in humans without a history of drug abuse, differences were noted across the two paradigms. For instance, although mazindol was not preferred over placebo in an experiment using a choice paradigm, in subjects that were trained to discriminate d-amphetamine from placebo, mazindol substituted as a discriminative stimulus (Chait, et al 1986, 1987). Since few drug discrimination studies have been conducted in humans, further research is necessary to determine the usefulness of drug discrimination methods to measure dependence potential (see paper by Bigelow).

DISCOVERING VULNERABILITY OR RISK FACTORS

Another goal of human drug self-administration studies has been to determine whether certain types of individuals are more likely to find certain drugs reinforcing. Such differences might indicate that individuals with these characteristics are vulnerable to drug abuse. The types of possible risk factors that have been studied in human drug self-administration studies have included demographic characteristics, psychological status, and drug use history.

In one series of studies, diazepam preference was assessed in several groups of subjects differing in levels of anxiety using the out-patient choice procedure previously described for amphetamine. The rationale of this experiment was that subjects who experienced a positive change in mood might be more likely to self-administer a substance that produced such a change (i.e., self-medication). Thus anxious subjects because of their high and presumably aversive levels of anxiety and who experience a decrease in their levels of anxiety when administered diazepam might be more likely than others to self-administer this drug. Subjects initially sampled the effects of 10 mg diazepam and placebo under double-blind conditions and then were given 5 independent opportunities to choose whether to self-administer diazepam or placebo. In normal volunteers, diazepam was not preferred to placebo (Johanson and Uhlenhuth 1980b). In subjects with moderate to severe levels of anxiety, diazepam preference was the same as in the control group, i.e., all subjects preferred placebo to a greater extent than drug despite the fact that diazepam decreased anxiety as measured by the Profile of Mood States (de Wit et al 1986).

However, in the group of subjects with the highest levels of anxiety and who wished to receive treatment, preference for diazepam was slightly increased However, placebo was still preferred on more than 50% of the choice opportunities even in this group (McCracken et al. in press). Thus it appears that a decrease in anxiety in subjects suffering from anxiety disorders is not sufficient for establishing diazepam as a reinforcer.

Previous drug history may also influence reinforcing properties. In an experiment by Johanson and her colleagues, the influence of previous alcohol consumption on diazepam preference was evaluated using a modified choice procedure (de Wit, et al. 1989). Experimental sessions were conducted within the laboratory and 4 mg doses of diazepam were available for a maximum of seven occasions during 4-hr evening sessions (total possible dose of 28 mg). Under these conditions, subjects with a history of greater but non-problematic alcohol consumption (approximately 11 drinks/week) preferred diazepam on more sessions than subjects with a history of light consumption (approximately 4 drinks/week). Furthermore, within sessions, the subjects who were moderate alcohol drinkers self-administered a greater proportion of the available doses.

Griffiths and his colleagues have also demonstrated that drug history can influence reinforcing properties. In their studies, subjects were recruited who had a history of sedative abuse and tested on a closed research ward. As previously shown with pentobarbital, diazepam was reliably self-administered across a range of experimental conditions (Griffiths, et al. 1979, Griffiths et al. 1980, Roache and Griffiths in press). This is in contrast to the results obtained by Johanson and her colleagues that indicated that diazepam was not a reinforcer in subjects who did not have a history of sedative abuse (Johanson and Uhlenhuth 1980b).

There are clearly many factors that could account for the influence of previous drug history on diazepam preference. Although additional research is required to determine the mechanisms accounting for the influence of drug history, these studies illustrate the possibility of evaluating risk factors within an experimental context in human subjects. Furthermore, the existence of populations of subjects that are more likely to self-administer a particular class of drug allows questions concerning relative dependence potential within this class to be addressed (Griffiths et al. 1984).

Animal research has clearly demonstrated that the reinforcing effects of drugs are not solely determined by their pharmacological properties but are the result of an interaction between the effects of the drug and the environmental conditions under which it is available. Therefore, environmental factors that increase drug self-administration may also be viewed as risk factors. However, few human drug self-administration studies have evaluated the ability of behavioral factors to modify the reinforcing properties of drugs (but also see Behavioral Treatments below). Early self-administration studies that were reviewed above (Bigelow, et al. 1976, Griffiths. et al. 1976) demonstrated that response requirements and minimum interingestion interval could influence the probability that sedatives would be self-administered. A mom recent example is the study described above by de Wit et al. (1989). As previously reviewed, under a choice paradigm where normal volunteers were allowed to choose between diazepam and placebo, placebo was preferred (Johanson and Uhlenhuth 1980b). However, when the experimental conditions under which diazepam was available were altered, it was found that diazepam preference drastically increased to over 50%. Unfortunately, the modifications that yielded an increase in reinforcing properties were numerous (laboratory setting, multiple doses available per session, subjects tested in a group context) so additional

research is necessary to determine what aspect of the environment accounts for the increase in reinforcing properties. Nevertheless, this study does indicate that the elucidation of environmental circumstances that enhance a drug's reinforcing properties and which therefore might constitute a risk factor is possible using human subjects.

EVALUATING POTENTIAL TREATMENTS

Pharmacological Treatments

Human drug self-administration paradigms are ideal for determining whether medications are capable of decreasing drug taking behavior. An excellent example of this approach is Mello et al. (1981). In their study, male heroin addicts resided on a research ward for a month. After an initial drug-free period, they were allowed to respond under a second-order schedule on a portable manipulandum to obtain either money or a dose of heroin. For 10 days, a total of 4 doses of 10 mg heroin was available every day with each injection separated by 6 hr. In order to obtain each dose, it took about 90 min of responding to complete the behavioral requirement under the second-order schedule. In three subjects that were treated with a narcotic antagonist, 50 mg/day naltrexone, few injections of heroin were self-administered. In contrast, subjects administered a placebo antagonist worked for a large proportion of the available heroin. A similar study was conducted by Mello et al. (1982) to evaluate the effectiveness of a mixed opioid agonist-antagonist, buptenorphine, to decrease heroin self-administration and similar results were found. That is, subjects maintained on gradually increasing doses from 0.5 to 8 mg/day buprenorphine over a 14 day period self-administered less of the available heroin than subjects who were medicated with a buprenorphine placebo. As the authors stated, the results from both of these studies not only demonstrate that naltrexone and buprenorphine may be useful medications for suppressing heroin abuse but they also indicate that human self-administration paradigms are useful for evaluating potential treatments. Given the difficulty of conducting long-term clinical trials of new pharmacological agents for the treatment of drug abuse, the approach of initially conducting laboratory studies using self-administration procedures is extremely appealing.

Laboratory studies may also be helpful in understanding the mechanisms underlying the usefulness of particular medications. With this in mind, Fischman and Foltin (1988) assessed the ability of desipramine which has been shown in clinical trials to be effective in the treatment of cocaine abuse to decrease cocaine self-administration. The ability of cocaine to maintain self-administration was evaluated both before and during a period of chronic desipramine treatment. The number of injections self-administered under these two conditions did not differ as might have been predicted from the clinical data However, the number of injections that the investigators were able to administer decreased when desipramine was being given because of cardiovascular toxicity. Furthermore, while subjects worked to obtain and requested nearly maximum levels of cocaine to self-administer, their verbal response to questions concerning their desire to self-administer cocaine indicated a decrease in craving. In addition, the subjective effects of cocaine were altered during the chronic desipramine period showing decreases in positive mood effects and increases in negative mood effects such as anxiety. Thus these results indicate that desipramine does produce alternations in the profile of cocaine's subjective effects which may be useful in treatment settings.

Behavioral Treatments

Behavioral approaches are also used to treat drug abuse and it is possible to evaluate their effectiveness in experimental settings. Stitzer and her colleagues evaluated the ability of alternative reinforcers to decrease methadone self-administration using outpatients being treated in a methadone maintenance clinic (Stitzer, et al. 1983). On two days of each week, subjects were offered the opportunity to choose between extra methadone doses and money. When the choice was between a $1 and drug, drug choice increased as dose of methadone increased. That is, when the dose was 1 mg, money was selected on about 80% of the opportunities whereas when the dose was 50 mg, drug was chosen exclusively. However, when the amount of the money option was increased to $5, the dose-response function for methadone was shifted downward and even when the extra dose available was 50 mg, only about 60% of the choices were for drug. This study illustrates that behavioral factors such as financial alternatives can alter drug self-administration. The results also show, however, that this ability to decrease drug intake is related to its dose. Both findings have important implications for designing behavioral treatment interventions.

In another study by Stitzer and her colleagues, these investigators evaluated the effects of two kinds of behavioral interventions on the self-administration of illicit drugs within a treatment context (Iguchi et al. 1988). Methadone maintenance patients who had tested positive for illicit drug use 70% of the time over the last 12 weeks were selected. Half of these subjects were given methadone take-home privileges contingent upon the submission of negative urines. The other half were also given contingent positive reinforcement (take-home medication) but were also punished if their urines were positive by a reduction in methadone dose. Both groups showed similar levels of success as defined by negative urines and remaining in the treatment program. However, the group that received punishment had higher drop-out rates from the program. The authors concluded that aversive consequences can influence treatment outcome by increasing the probability that patients who do not respond to treatment will remove themselves from the situation, thus limiting the ability of therapists to intervene any further.

DESCRIBING CONSEQUENCES OF DRUG SELF-ADMINISTRATION

Toxic effects which are seen in drug abusers are often difficult to attribute to the drug itself as many other factors contribute to manifest toxicity in the environment outside the laboratory. Thus, measuring the effects of drugs that are self-administered in a laboratory setting offers an opportunity to evaluate the contribution of the drug itself to changes in health and behavior of drug abusers. Unfortunately, few experimental studies have been conducted even in animal subjects and even fewer with humans. For humans, this is largely due to the unwillingness to expose subjects to the doses that are likely to produce toxic consequences. Thus, while the effects of drugs on physiological functioning are routinely measured in human drug self-administration studies, the doses are kept necessarily low. Nevertheless, while these studies may not reveal actual toxicity, they may show that drugs alter the functioning of certain physiological systems; it can then be assumed that toxicity is likely at higher doses (Fischman and Foltin 1989).

In addition to physiological alterations, some studies have also evaluated the effects of drugs at self-administered doses on mood. Griffiths et al. (1980), for instance, showed that hostile behavior and feelings of dysphoria were more likely on days when subjects self-administered diazepam than when pentobarbital was taken. These

findings clearly have implications for the use of these drugs in a treatment context. However, they are also relevant in situations where these drugs are being abused because they indicate that benzodiazepines may disrupt social interactions.

Another example of how drug self-administration studies can be useful for evaluating the consequences of drug use is from a study by Mendelson et al (1976). Heavy and casual marijuana smokers resided on a research ward for one month and for 2 1 days during this period were allowed to respond on a portable manipulandum under a fixed interval schedule to earn marijuana cigarettes or money. Approximately 30 minutes of work was required to earn 1 cigarette or $0.50. Relative to days when drug was not available, marijuana even at relatively high levels did not disrupt performance. Further, patterns of responding within days indicated that rate of responding correlated with rate of smoking. These data suggested, therefore, that marijuana did not produce an "amotivational syndrome." However, in a further analysis of the data, Mendelson et al (1976), showed that there was an inverse relationship between amount of marijuana consumed and the rate of responding on the following day. While there are clearly many possible interpretations of these data, this study does indicate that self-administration studies may be useful for evaluating changes in performance that occur at doses that are self-administered

CONCLUSION

This report has reviewed the types of research questions that can be answered in human self-administration studies. The goals illustrated are not exhaustive but include a wide enough sample to demonstrate the utility of these procedures. Furthermore, by using specific examples, it is hopefully possible for those unfamiliar with the research area to appreciate the range of conditions under which evaluations of the reinforcing properties of drugs can be made with human subjects.

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AUTHOR

Chris-Ellyn Johanson, Ph.D.
Department of Psychiatry
Unifcrmecl Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814

Behavioral and EEG Studies of Acute Cocaine Administration: Comparisons with Morphine, Amphetamine, Pentobarbital, Nicotine, Ethanol and Marijuana

Scott E. Lukas, Jack H. Mendelson, Leslie Amass and Richard Benedikt

Over the past five years we have been engaged in studies designed to determine the electrophysiological correlates of drug-induced behavioral states. Since the basic mechanism of drug-seeking behavior is not completely understood, the identification of such correlations between mood states and electrophysiological activity can be very important in relating these changes to the reinforcing properties of various drugs of abuse.

Measures of brain electrical activity have been useful in characterizing various naturally-occurring behavioral states such as sleep and wakefulness, but electrophysiological correlates of drug-induced states such as euphoria have been difficult to obtain. A major problem with detecting such a relationship between brain electrical activity and drug-induced mood changes is the difficulty associated with accurately measuring changes in subjective mood states without introducing EEG artifacts. Questionnaires and visual analog scales alter a subject's levels of alertness which, in turn, affects the EEG (Otto, 1967; Matousek and Petersen, 1983). Therefore, we have developed a nonverbal instrumental device for recording continuous changes in drug-induced mood states which requires a minimum of effort and, as such, does not alter the subjects' level of alertness (Lukas *et al.,* 1986a,c.)

METHODS

Adult male and female volunteers provided informed consent to be prepared with scalp EEG electrodes and intravenous catheters for blood withdrawal. Subjects who received drugs intravenously did not have blood withdrawn. Subjects served as their own controls and received placebo as well as all doses of a drug. The following drugs and doses were tested: morphine (5, 10 or 20 mg, i.v.), amphetamine (5, 10 or 20 mg. i.v.), pentobarbital (50, 100 or 200 mg. i.v.), nicotine (0.75, 1.5 or 3.0 mg, i.v.), cocaine (32, 64 or 96 mg, i.n.), ethanol (0.35 or 0.7 g/kg, p.o.) or marihuana (1.26 or 2.53% Δ^9-THC, i.h.)

Continuous measures of drug-induced behavioral states were obtained by having the subjects operate an instrumental joystick device (Lukas *et al.,* 1986c.) In addition to providing data indicating the onset, duration and offset of drug effects, the subjects could push two different buttons to indicate euphoric or pleasurable and dysphoric or unpleasant experiences. All joystick responses were recorded on the polygraph paper directly below the EEG recordings.

Morphine, amphetamine, pentobarbital and nicotine were administered as slow injections over 120 seconds. Marihuana cigarettes were smoked using a modified water pipe device and using a specific series of inhale/hold/exhale instructions. Ethanol solutions were pumped through a tube that rested in the subjects' mouth at a rate of 23 ml per minute. Cocaine was administered intranasally as an aerosol. Two computer-controlled, one-second bursts (one in each nostril) were provided with a five second inter-burst interval. A customized air brush device was used to deliver the cocaine(Figure 1.) These techniques were used so that subjects could remain seated with their eyes closed and their left hand on the joystick device during drug administration. Thus, subject movements were kept to a minimum in order to eliminate EEG artifacts.

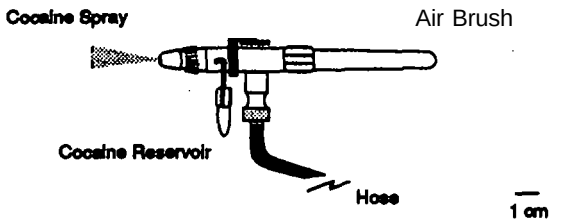
EEG activity was recorded on a polygraph and on FM magnetic tape for subsequent off-line computerized power spectral analysis. Data were recorded continuously from thirty minutes before to two hours after drug administration. A digital time code (which was copied onto the polygraph tracing) was also recorded on tape to aid in the identification of specific EEG epochs that were associated with abrupt behavioral changes as indicated by joystick responding (Lukas *et al.,* 1986b.)

Discrete samples of EEG activity that occurred while subjects reported feeling good or euphoric were digitized and subjected to Fast Fourier Transformations in order to generate the corresponding power spectra. In addition, the data from some subjects who received ethanol or cocaine were further analyzed using EEG topographic mapping techniques. These mapping techniques assembled information from multiple EEG leads by simultaneously creating power spectral arrays from all electrode sites. The values between electrode sites were computed with a three-point linear interpolation algorithm using the activity from the nearest three electrodes. The data were then combined into a composite color-coded (Duffy *et al.,* 1979; Lukas *et al.,* 1989) map which provided an overall view of brain electrical activity.

RESULTS

Subjects reported drug-induced behavioral changes by operating an instrumental joystick device. Multiple paroxysmal bursts of euphoria occurred within a few minutes of drug administration and each one typically lasted 1-10 minutes while subjects continued to detect the drugs' unique pharmacological effects (Figure 2.) The total duration of euphoria was dose-related. Dysphoric responses after nicotine and ethanol were frequently reported immediately after the highest doses, but were usually replaced by euphoria within a few minutes. When questioned after the studies were over, subjects were very accurate in correctly identifying the intermediate and higher drug doses.

A microanalysis of the EEG after low and intermediate doses of all drugs tested revealed that EEG alpha activity was significantly increased during these episodes of drug-induced euphoria. Further analysis of some records using topographic mapping techniques revealed that the increase in EEG alpha activity after ethanol and cocaine was most prominent over the occipital area. In addition, ethanol-induced euphoria was associated with a significant increase in EEG alpha over more frontal and parietal areas while cocaine-induced increases remained over occipital and parietal areas.



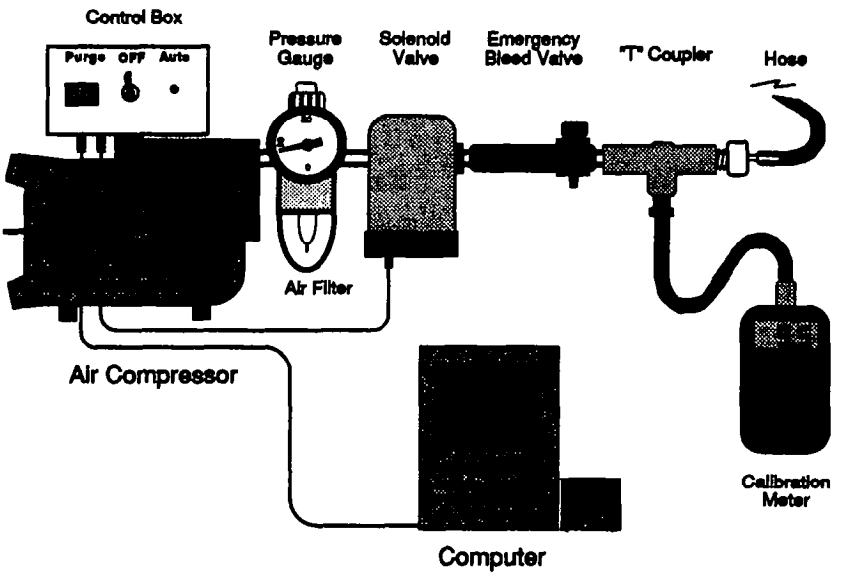


Figure 1

Intranasal drug delivery device for providing a computer-controlled metered dose of cocaine as a fine spray. The air brush is supported by a flexible metal arm which permits the subjects to receive the cocaine without moving. The air compressor and valve system are located outside the experimental chamber so that the subject is not disturbed during their operation.

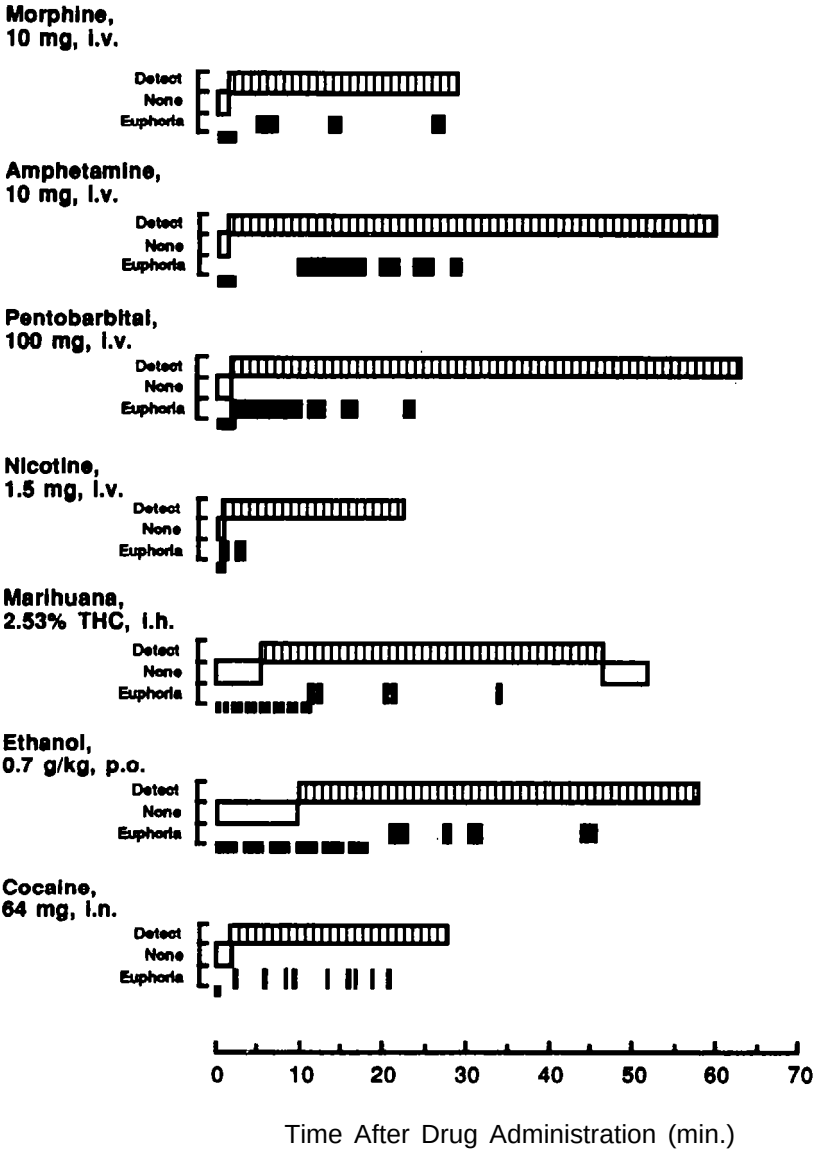


Figure 2

Behavioral profiles after acute drug administration. Changes in behavior were continuously recorded via an instrumental joystick device. Data are from individual, representative subjects. Shaded blocks below each profile indicate the actual drug delivery times.

DISCUSSION

One of the more interesting findings in the present report is that a number of drugs from various distinct pharmacological classes produced a similar EEG and behavioral response. It should be noted that the highest drug doses typically produced immediate and profound stimulation or depression, which may have concealed the EEG alpha response observed after the lower doses. This was most evident after the higher doses of cocaine in which behavioral stimulation was associated with EEG dyschronization (beta activity) and pentobarbital which resulted in behavioral depression and EEG slow wave activity. However, the low and intermediate doses all resulted in paroxysmal bursts of euphoria, which were associated with increases in EEG alpha activity. EEG alpha activity was not increased during controlled joystick responding, so it is unlikely that this neurophysiologic response is movement-related.

The relatively brief nature of these drug-induced euphoric episodes is difficult to explain. While plasma drug levels were not obtained for all drugs, it does appear that both a threshold level and a relatively rapid rate of increase in plasma drug levels is necessary for euphoria to occur. However, these data only reflect the concentration of drug in blood and not in the central nervous system.

The neurobiological significance of increased EEG alpha activity during drug-induced euphoria is not completely understood. Increases in EEG alpha activity are frequently associated with pleasurable, free-floating and extremely relaxed states (Lindsley, 1952; Brown, 1970; Wallace, 1970; Matejcek, 1982) that are not unlike those associated with transcendental meditation (Wallace, 1970.) The covariance between increased EEG alpha activity over the entire scalp and subjective reports of euphoria suggests that this neurophysiologic response may be associated with drug-induced reinforcement. These data further suggest that drugs of abuse may share a common mediator that is associated with reinforcement. This interpretation supports the contention of Mello (1983) that drug abuse is a form of *stimulus* administration and that the direction of change is relatively unimportant while the speed and magnitude of change dictate the response.

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ACKNOWLEDGEMENTS

Supported by NIDA Grants DA 03994 and DA 04059, Research Scientist Development Award DA 00115 and Research Scientist Award DA00064.

AUTHORS

Scott E. Lukas, Ph.D., Jack H. Mendelson, M.D., Leslie Amass, M.A. and Richard Benedikt, M.D.

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, Massachusetts 02178

Plasma Delta-9-THC Levels as a Predictive Measure of Marijuana Use by Women

Jack H. Mendelson, Nancy K. Mello, Siew Koon Teoh, Barbara W. Lex, Scott E. Lukas and James Ellingboe

A number of studies carried out at our Center examined marijuana-induced alterations in hormonal homeostasis in women (Mendelson et al., 1984, Mendelson et al., 1985 a,b). However, plasma delta-9-THC levels were not measured in these studies, and we have been unable to locate any reports which describe relationships between plasma delta-9-THC levels and marijuana smoking by women. The purpose of this study was to determine plasma delta-9-THC levels in women prior to, during and following a period of marijuana self-administration on a clinical research ward.

METHODS

Subjects

Sixteen adult female volunteers (average age 26, range 21 to 34 years) with a history of regular marijuana use, gave informed consent for participation in studies to evaluate the effects of marijuana smoking on reproductive function in an inpatient clinical research study. Volunteer subjects with a mean of 13.5 years of education were recruited through advertisements in local newspapers. Subjects were fully informed about the nature and duration of each phase of the study and were told that they could withdraw at any time. Each woman was in good health as determined by clinical and laboratory examinations. Urine screens for drug use other than marijuana and pregnancy tests were performed before admission to the research ward and all were negative.

Sequence of Drug Conditions

After admission to the research ward, subjects were drug-free for 7 days. Marijuana cigarettes were available for 21 days. After the period of marijuana availability, subjects remained on the ward for an additional 7 days, under drug-free conditions, to evaluate the time course and severity of any marijuana-related abstinence syndromes (cf. Mendelson et al., 1984).

Marijuana Cigarettes

All marijuana cigarettes were obtained from NIDA in lot standard dosage form. Maximal standardization and the equivalent dosage and "draw" characteristics of these cigarettes were ensured by machine rolling. Each cigarette weighed approximately 1 g and contained 1.83% of Δ9-tetrahydrocannabinol as assayed by NIDA. Details of the Soxhlet and modified Lerner extraction procedures and the gas chromatogmphic assay procedure are available from NIDA.

Marijuana and Money Acquisition Procedures

Operant techniques were used to provide an objective and quantitative measure of performance for 2 alternative reinforcers, marijuana and money. Subjects could work for money at a simple operant task throughout the study. The opportunity to work for money was intended to encourage subjects to remain in the study during the 14 days when marijuana was not available. Points earned at the operant task could be accumulated for money, payable at the end of the study, or could be exchanged for marijuana cigarettes during the period of marijuana availability. Patterns of operant acquisition of marijuana by women have been reported previously (Mello and Mendelson 1985).

Blood Sampling Procedures

Blood samples for THC analyses were obtained on admission to the ward; again after 7 drug-free days; on alternate days during the 21 day period of marijuana cigarette availability; and 1 and 7 days following cessation of marijuana use. Blood samples were taken between 8 and 9 a.m. Most recent marijuana smoking for each subject prior to blood sampling occurred between 11 p.m. and 1 a.m.

RADIOIMMUNOASSAY OF DELTA-9-TETRAHYDROCANNABINOL (DELTA-9-THC) IN PLASMA

Delta-9-THC was analyzed by radioimmunoassay using kits obtained from the Research Triangle Institute, where they were prepared with support from Contract No. 271-81-3828 from the National Institute on Drug Abuse. The kits provided I-125-labeled delta-8-THC, rabbit anti-delta-9-THC serum, an immunobead second antibody reagent, blank human plasma, delta-9-THC standards in human plasma, blank human plasma and delta-9-THC controls. Plasma samples were analyzed in duplicate, following the protocol supplied with the kits. In our laboratory the sensitivity was 1.0 ng/ml. Intraassay CVs, calculated from duplicates of all samples, including those with very low delta-9-THC concentrations, averaged 21.4%. Interassay CVs, determined using controls supplied by the Research Triangle Institute, were 12.6% for the high control (31.2 ng/ml mean value) and 12.4% for the low control (8.0 ng/ml mean value). According to information from the Research Triangle Institute, cross reactivities for other cannabinoids in this assay were as follows: delta-9-THC 100%. 11-nor-9-carboxy-delta-9-TCH 0.6%, 11-hydroxy-delta-9-THC 21%. delta-8-THC > 70%. cannabinol 30%. cannabidiol 0.23%. 8-alpha-hydroxy-delta-9-THC 10% and 8 beta-hydroxy-delta-9-THC 7%. In order to ascertain the accuracy of our radioimmunoassay procedures we forwarded selected plasma samples to Dr. Roger Foltz (Utah Toxicology Laboratory) for confirmatory gas chromatographic analysis. Plasma delta-9-THC values obtained with the RIA procedure were higher than those determined by gas

chromatography (Wilcoxon Matched Pairs Test t [n=21] = 47.0, z = 3.482, p = 0.0164). but the linear correlation between the 2 methods was highly significant (r^2 - 0.992).

RESULTS

The 16 women who participated in this study were classified into 3 groups based upon the actual number of marijuana cigarettes smoked on the research ward. Table 1 presents smoking behavior, plasma delta-9-THC levels and demographic data for the 3 groups of subjects. Four subjects in group 1 smoked an average of 4.7 marijuana cigarettes per day on the research ward. Subjects in group 2 smoked an average of 2.7 marijuana cigarettes per day on the research ward, and subjects in group 3 smoked an average of 0.4 marijuana cigarettes per day during the study. There were no significant differences between the ages of the subjects or their level of education. Subjects in group 1 reported an average past history of marijuana use of 15.5 years which was approximately twice as long as the duration of marijuana use reported by subjects in groups 2 and 3 (7.7 and 7.6 years, respectively). Subjects in group 1 reported an average use of marijuana 35 times per month and subjects in groups 2 and 3 reported average use of 13 and 7.6 cigarettes per month, respectively. No detectable levels of plasma delta-9-THC were found for the 5 subjects in group 3. In contrast, peak delta-9-THC levels for the group 1 subjects were 75.8 ng/ml and 32.9 ng/ml for subjects in group 2.

TABLE 1

Group	$\bar{x}$ Use Per Study Day	$\bar{x}$ Peak Δ^9 THC (ng/ml)	$\bar{x}$ Years of Use	$\bar{x}$ Times Used Per Month
1 (N=4)	4.7	75.8	15.5	35
2 (N=7)	2.7	32.9	7.7	13
3 (N=5)	0.4	N.D.	7.6	7.6

Individual values for peak delta-9-THC levels for group 1 and 2 subjects as well as mean levels are shown in figure 1. Peak delta-9-THC levels were significantly greater for group 1 as compared to group 2 subjects ($P < .05$). Figure 2 shows individual and mean delta-9-THC levels for group 1 and 2 subjects on study days 2 and 7. Mean plasma delta-9-THC levels were greater for group 1 subjects on day 2, however, these differences did not achieve statistically significant values. On day 7, when subjects had been abstinent from all marijuana use since their admission to the research ward, plasma delta-9-THC levels were similar for both groups of subjects. Figure 3 shows plasma

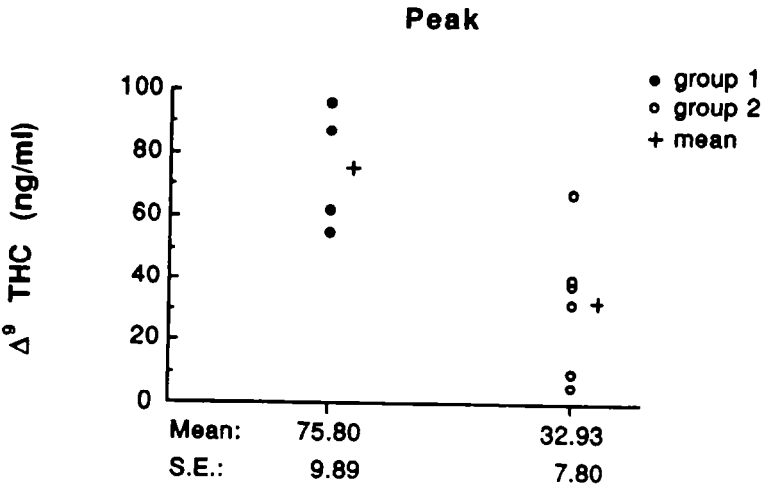

FIGURE 1

delta-9-THC levels on day 30 (the first day following cessation of marijuana use on the research ward) and on day 35. No statistically significant differences were observed for plasma delta-9-THC levels for the 2 groups of subjects.

DISCUSSION

Relationships between marijuana smoking and plasma THC levels observed in this study are consistent with the pharmacokinetic profile of marijuana effects described by Hunt and Jones (1980). A good correlation was found between the number of marijuana cigarettes smoked and peak plasma delta-9-THC levels. Women who reported the highest level of marijuana smoking prior to participation in the study smoked the greatest amount of marijuana during the research ward study. Those women who reported smoking the least amount of marijuana prior to the study smoked the smallest number of cigarettes during the study in comparison to other subjects. Thus verbal reports by women of past history of marijuana use, at least when obtained in the context of a research program, appear to be highly reliable when confirmed by observations of marijuana smoking under controlled investigator-observed conditions.

Women who reported the longest duration and greatest use of marijuana prior to the study, smoked the largest number of cigarettes on the research ward and had the highest peak plasma delta-9-THC levels. Moreover, these women also had higher plasma delta-9-THC levels when they entered the study in contrast to other subjects. These data suggest that plasma delta-9-THC levels may be a reliable marker for not only past history of marijuana use but also for predicting future smoking behavior when marijuana cigarettes are freely available. However, lack of significant differences between plasma delta-9-THC levels following 5-7 days of abstinence in women who smoked various amounts of marijuana suggest that plasma delta-9THC levels would be a poor marker for previous marijuana use approximately 1 week after cessation of smoking. If

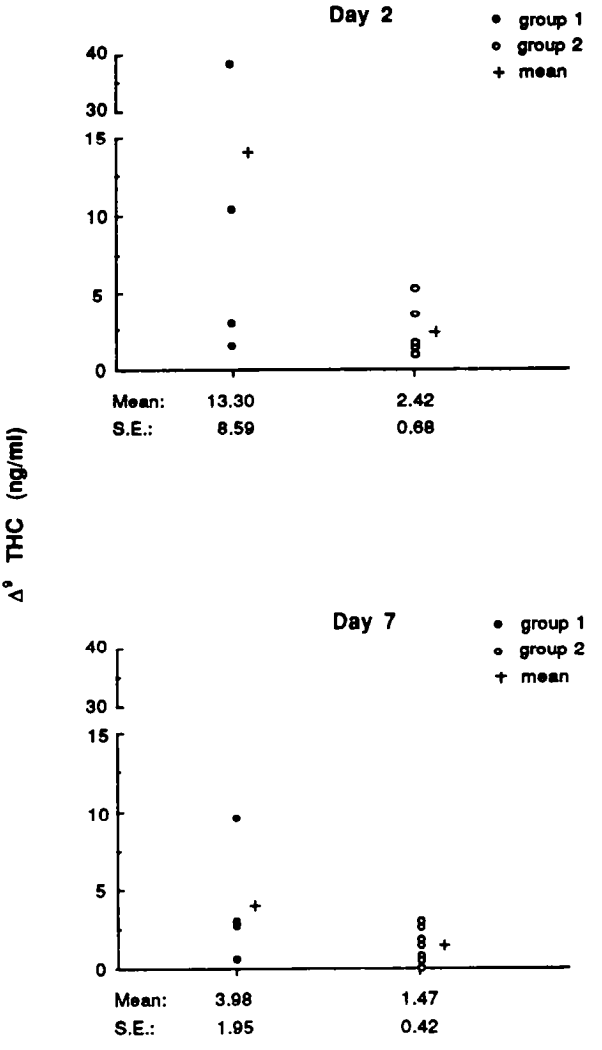
Day 2
group 1
group 2
mean
40
30
15
10
5
0
Mean: 13.30 2.42
S.E.: 8.59 0.68
Day 7
group 1
group 2
mean
40
30
15
10
5
0
Mean: 3.98 1.47
S.E.: 1.95 0.42
Δ⁹ THC (ng/ml)

FIGURE 2

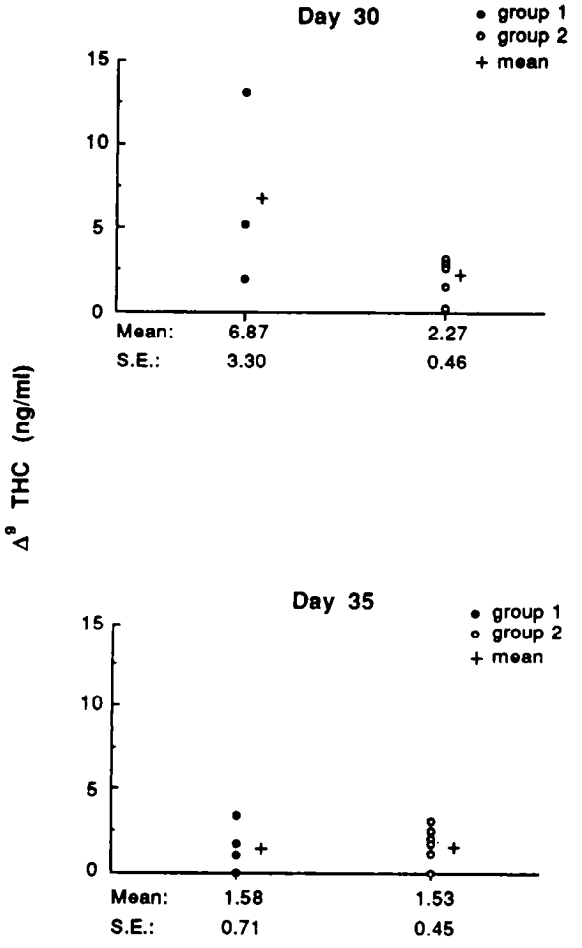
Day 30
group 1
group 2
mean
15
10
5
0
Mean:
6.87
2.27
S.E.:
3.30
0.46
Day 35
group 1
group 2
mean
15
10
5
0
Mean:
1.58
1.53
S.E.:
0.71
0.45
Δ⁹ THC (ng/ml)

FIGURE 3

THC and THC metabolite levels in urine specimens are related to plasma delta-9-THC levels, similar problems would be encountered in interpreting the significance of urine testing for either determining past history of marijuana use or predicting future marijuana smoking behavior.

Plasma delta-9-THC levels were undetectable in those women who smoked less than 1 marijuana cigarette (average 0.4 marijuana cigarettes) per day during the study. These women reported they smoked an average of 7.6 marijuana cigarettes per month and none had detectable plasma delta-9-THC levels on admission to the study. Thus the women who used marijuana least frequently had the least probability of detection of plasma delta-9-THC levels prior to or following the study. If plasma delta-9-THC levels are correlated with long term presence. or absence of delta-9-THC in urine, similar false negative findings might be anticipated from urine test results. We have been unable to locate any study which has attempted to correlate plasma delta-9-THC levels following observed marijuana use with the magnitude and duration of positive results in urine screening tests. Since it has been argued that false positives in marijuana urine screening may be more likely to occur than false negatives, such studies may be of assistance in determining the effectiveness of urine tests in pre-employment screening and work place settings.

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ACKNOWLEDGEMENTS

This study was supported by grants AA06252 from the National Institute on Alcohol Abuse and Alcoholism and DA00064 and DA00101 from the National Institute on Drug Abuse.

AUTHORS

Jack H. Mendelson. M.D., Nancy K. Mello, Ph.D., Siew Koon Teoh, M.D., Barbara W. Lex, Ph.D., Scott E. Lukas. Ph.D., James Ellingboe, Ph.D.

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02178

Opioid Receptors and Ligands

Hans W. Kosterlitz, Alistair D. Corbett and Stewart J. Paterson

INTRODUCTION

One of the main problems of our understanding of the mode of action of the endogenous opioid peptides is the multiplicity of the fragments of pro-opiocortin, pro-enkephalin and prodynorphin. Furthermore, none of the fragments interact with only one of the μ-, δ- and κ- sites of the receptors (Kosterlitz and Paterson, 1985). The complexity of the system has recently been aggravated by the finding that the non-peptide morphine is present in low concentrations in animal tissue where it can be biosynthesized from its non-morphinan precursor reticuline (Donner et al, 1986, 1987; Weitz et al., 1986, 1987). Its possible physiological significance in animal tissue is still uncertain (Kosterlitz, 1987).

It is important to note that the binding interaction of a molecule may or may not lead to a biological response of either excitatory or inhibitory activity. Pharmacologically, such activity would indicate that the response is that of an agonist. An antagonist compound would also bind to the receptor but block its excitatory or inhibitory response.

A difficulty of the interpretation of the physiological effects of the endogenous opioid peptides is due to the fact that almost all are degraded by peptidases. It is therefore necessary either to use antipeptidases or synthetic peptidase-resistant analogues, the action of which may be different from the endogenous peptides.

BIOASSAYS OF OPIOID μ-, δ- AND κ- RECEPTORS IN ISOLATED TISSUE PREPARATIONS

While a final understanding of the mode of action of opioid peptides or non-peptide opioids has to await investigations in vivo, the pharmacological effects of opioid compounds and their physiological properties are often more readily analysed in excised tissues. By such an approach, it is possible to decide whether a synthetic opioid has agonist or antagonist properties

or a combination of both. Such a differentiation is not possible in binding assays on homogenized brain membranes.

In the bioassays used in this section, the opioid peptides or non-peptides have high selectivity for one of the μ-, δ- or κ-receptors. The assay tissues are from five different species which have opioid receptors of one, two or three subtypes (TABLE 1). The opioid receptors of the rabbit vas deferens interact with κ-ligands, e.g. dynorphin A and U-69,593, those of the hamster vas deferens with δ-ligands, e.g. [D-Pen^2,D-Pen^5] enkephalin, those of the rat vas deferens mainly but not solely, with μ-ligands, e.g. morphine and [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin and ß-endorphin those of the guinea-pig ileum with μ- and κ-ligands, e.g. [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin, dynorphin A and U-69,593, and, finally, those of the mouse vas deferens interact with all three μ-, δ- and κ-ligands (Kosterlitz and Paterson, 1985) .

TABLE 1

Opioid Receptor Activity (37°C) in Rat Vas Deferens (RVD), Hamster Vas Deferens (HVD), Rabbit Vas Deferens (LVD), Guinea Pig Ileum Myenteric Plexus (GPI) and Mouse Vas Deferens (MVD)[a]

	LVD (κ)	HVD (δ)	RVD (μ)	GPI (μ+κ)	MVD (μ+δ+κ)
Morphine	0	0	A	A	A
[D-Ala^2,MePhe^4,Gly-o1^5]enkephalin	0	0	A	A	A
ß-Endorphin	0	A	A	A	A
[Met]- and [Leu] enkephalin	0	A	A	A	A
[D-Pen^2,D-Pen^5]enkephalin	0	A	0	(A)	A
Dynorphin. A	A	(A)	0	A	A
U-69,593[b]	A	(A)	0	A	A
Naloxone	ANT	ANT	ANT	ANT	ANT
(-)-Bremazocine	A	ANT	ANT	A	A
(-)-Ethylketazocine	A	ANT	ANT	A	A

[a] 0 = no effect, A = agonist, ANT = antagonist. Antipeptidases were present for assay of [Met]- and [Leu]enkephalin and dynorphin A. (Kosterlitz 1985; Kosterlitz and Paterson 1985; McKnight et al., 1985; Paterson et al., 1984)

[b] Lahti et al., 1985. (5α,7α,8ß)-(+)-N-methyl-N(7-(l-pyrrolidinyl)-l-oxaspiro-[4,5]dec-B-yl)-benzeneacetamide

There are unsolved problems concerning the physiology and pharmacology of opioid compounds. Firstly, almost all endogenous opioid peptides are liable to degradation by peptidases and, secondly, potent selective agonists have only recently become available.

TABLE 2

Binding Affinities of μ-, δ- and κ-Opioid Antagonists and Their Relative Binding Affinities in Homogenates of Guinea Pig Brain. Antagonist Activity (K_e)

	Affinity (K_i, nM)$^{-1}$	Relative Affinity μ	δ	κ	Antagonist[d] (K_e, nM)
CTOP[a]	0.52(μ)	>0.998	-	-	16.1
Naltrindole[b]	8.3 (δ)	0.01	0.98	0.01	0.11
Norbinaltorphimine[c]	2.9 (κ)	0.02	0.03	0.95	0.14

[a] Hawkins et al., 1989. [b] Portoghese et al., 1988

[c] Portoghese et al., 1987

[d] Selective agonist for the u-site, [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin (GPI), for the δ-site, DPDPE (MVD), and for the κ-site, U-69,593 (GPI)

The new antagonist for the κ-site, norbinaltorphimine (Portoghese et al., 1987), has considerable selectivity for the K-receptor but appears to be degraded in brain tissue (Birch et al, 1987). Its properties will be discussed in a later section. One of the selective δ-antagonists, ICI 174,864, is highly selective but is of low potency (Cotton et al., 1984). It is now superseded by the potent and selective δ-antagonist naltrindole (Portoghese et al., 1988). Recently, a selective u-antagonist, CTOP, has become available (Hawkins et al., 1989) but its antagonist potency (K_e = 16 nM) is lower than that of the δ-antagonist of naltrindofe (K_e = 0.11 nM) or that of the K-antagonist norbinaltorphimine (K_e = 0.14 nM). (TABLE 2).

It is important to be aware of the fact that ligands may be agonists at opioid receptors in some tissues but antagonists in others. Ethylketazocine and bremazocine are examples of this behaviour, as they are κ-agonists in the guinea-pig ileum and the vasa deferentia of the mouse and rabbit but u-antagonists in the vas deferens of the rat and δ-antagonists in the vas deferens of the hamster. (TABLE]).

BINDING ASSAYS OF μ-, δ- AND κ-LIGANDS IN MEMBRANES OF GUINEA PIG BRAIN

The affinity of a ligand is given as its binding affinity constant, $(K_i, nM)^{-1}$, which is the reciprocal of its inhibition constant. (TABLES 3-5). In addition, it is useful to determine the relative binding affinities by the ratio K_i^{-1} for μ, δ or κ/K_i for $\mu + K_i^{-1}$ for $\delta + K_i^{-1}$ for κ), the maximum being 1.00. The temperature of the binding assays was 25°C when peptidase resistant compounds were used and 0°C when peptides that are sensitive to enzyme activity were used.

TABLE 3

Binding Affinities of u-Selective Opioids and Their Relative Binding Affinities for the μ-, δ- and κ-sites in Homogenates of Guinea Pig Brain [a]

	μ-Affinity $(K_i, nM)^{-1}$	Relative Affinity μ	δ	κ
[Met]enkephalyl-Arg-Arg-Val-NH_2	16.7	0.77	0.03	0.20
[Met]enkephalyl-Arg-Arg-Val-Gly-Arg-Pro-Glu-Trp-Trp-Met-Asp-Tyr-Gln (BAM 18)	3.4	0.68	0.06	0.26
[Met]enkephalyl-Arg-Phe	0.29	0.60	0.36	0.04
ß-Endorphin	0.49	0.52	0.45	0.03
Morphine	0.56	0.97	0.02	0.01
Tyr-D-Arg-Phe-Lys-NH_2	0.28	0.998	0	0.002

Relative binding affinities at the μ-, δ- and κ-sites are: K_i^{-1} for μ, δ or $\kappa/(K_i^{-1}$ for $\mu + K_i^{-1}$ for $\delta + K_i^{-1}$ for κ)

[a] Hurlbut et al., 1987; Kosterlitz 1985; Kosterlitz and Paterson 1985; Paterson et al., 1984; Schiller et al., 1989.

In TABLE 3 four peptides are shown that, at the u-site, have relative affinities varying between 0.52 and 0.77. Two of these are [Met]enkephalins extended at the C-terminus with -Arg-Arg in positions 6 and 7. [Met]enkephalyl-Arg-Arg-Val-NH2 is of particular interest since it has the very high affinity

of 16.7 nM^{-1}. The relative affinities of this peptide and its possible precursor, BAM 18 (Hurlbut et al., 1987) are 0.77 and 0.68 at the u-site, 0.20 and 0.26 at the K-site and very low at the δ-site. In contrast, [Metlenkephalyl-Arg-Phe and ß-endorphin have much lower binding affinities of 0.29 nM^{-1} and 0.49 nM^{-1}. Their relative affinities are 0.60 and 0.52 at the u-site, 0.36 and 0.45 at the δ-site and very low at the K-site.

The binding pattern of morphine is very different. As discussed in the Introduction, morphine is an endogenous compound, but is present only in low concentrations. Its binding affinity is of an average order but it is of particular interest that this plant opioid has the very high relative u-affinity of 0.97 which is much higher than the relative affinities of any of the known endogenous μ-opioids in animal tissue.

Since none of the endogenous peptides is sufficiently selective for the u-site, it was important to obtain synthetic compounds. Tyr-D-Arg-Phe-Lys-NH_2 (TABLE 3) and [D-Ala^2,$MePhe^4$, Gly-ol^5]enkephalin (TABLE 1) fulfil this purpose.

TABLE 4

Binding Affinities of δ-Selective Opioids and Their Relative Binding Affinities for the μ-, δ- and κ-Sites in Homogenates of Guinea Pig Brain[a]

	δ-Affinity (K_i, nM)$^{-1}$	Relative Affinity μ	δ	κ
[Leu]enkephalin	0.85	0.06	0.94	0
[Met]enkephalin	1.10	0.09	0.91	0
[D-Ala^2,D-Leu^5]enkephalin	0.74	0.10	0.90	0
[D-Pen^2,D-Pen^5]enkephalin	0.37	0.004	0.996	0

[a] Kosterlitz and Paterson 1985; Paterson et al., 1984

The endogenous δ-ligands, [Met]enkephalin and [Leu]enkephalin have relative affinities of 0.91 and 0.94 but the low values of 0.09 and 0.06 at the μ-site (TABLE 4). These high values at the δ-site should be compared with the corresponding maximum values for the peptide μ-ligands of 0.77 (TABLE 3) and for the κ-ligand of 0.83 (TABLE 5) The availability of an even more selective δ-ligand, [D-Pen^2,D-Pen^5]enkephalin has become important, as is the already mentioned δ-antagonist natrindole (TABLE 2).

TABLE 5

Binding Affinities of κ-Selective Opioids and Their Relative Binding Affinities for the μ-, δ- and κ-Sites in Homogenates of Guinea Pig Brain[a]

	κ-Affinity (K_i, nM)$^{-1}$	Relative Affinity μ	δ	κ
Dynorphin A	8.7	0.13	0.04	0.83
Dynorphin B	8.5	0.14	0.03	0.83
α-Neo-endorphin	5.1	0.10	0.23	0.67
Dynorphin A (1-8)	0.75	0.22	0.16	0.62
[D-Pro^{10}]dynorphin A (1-11)[a]	34.7	0.05	0.01	0.94
U-69,593	0.74	0.001	0	0.999
PD 117302[b]	1.72	0.003	0	0.997

[a] Kosterlitz 1985; Kosterlitz and Paterson 1985; Paterson et al., 1984

[b] Birchmore et al., 1987. (+)-trans-N-methyl-N-[2-(l-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide

With regard to κ-ligands, there are no endogenous peptidase-resistant compounds of high potency, with the possible exception of dynorphin A (1-17). Dynorphin A (1-17) and dynorphin B (1-13) and a-neo-endorphin have high affinities at the K-sites (5.1-8.7 nM^{-1}). In contrast, the fragment dynorphin A (1-8), present in many areas of the nervous system in higher concentrations than dynorphin A (1-17), has a much lower affinity at the K-site (0.75 nM^{-1}) and is also much less selective. The physiological significance of this difference between the two peptides is still not understood (TABLE 5).

Non-peptide ligands with a very high degree of selectivity for the κ-binding,site are U-69,593 and PD 117,302 with relative affinities at the K-site of more than 0.99. Another interesting compound is [D-Pro^{10}]dynorphin A (1-11) which has the highest affinity to the κ-site so far observed but its relative affinity at the κ-site is somewhat lower than the values

obtained with the two compounds,

CONCLUSIONS

An investigation of the present state of our knowledge of the opioid receptors and their ligands confirms that analyses of their mode of action is now increasingly complex. The synthesis by Portoghese and his group of compounds which are selective δ-antagonist and almost selective κ-antagonists is of high significance. We may expect that our understanding of the mode of action of endogenous opioid fragments will be much facilitated.

The discovery by the groups of Goldstein and of Spector that morphine is an endogenous opioid, although present only in low concentrations, may not only be of pharmacological but also of physiological significance. Whatever the results of future experiments may indicate, it is interesting that morphine is the only known member of the morphinan group having such a highly selective μ-opioid ligand. The known endogenous opioid peptides are much less selective in this respect.

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ACKNOWLEDGMENTS

Supported by grants from the Medical Research Council and the National Institute on Drug Abuse (DA-00662).

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Hans W. Kosterlitz, M.D., Ph.D.
Alistair D. Corbett, Ph.D.
Stewart J. Paterson, Ph.D.
University of Aberdeen
Unit for Research on Addictive Drugs
Marischal College
Aberdeen, AB9 1AS, Scotland

The Role of Opioids in Analgesia and Gastrointestinal Function

Thomas F. Burks

Two of the most striking pharmacological effects of opioids in animals and humans are those affecting sensory perception of pain and those affecting gastrointestinal function. The antinociceptive or analgesic effects of opioids occur primarily from their actions in the brain and spinal cord. The antidiarrheal effects of opioids result both from central nervous system and enteric nervous system sites of action (Parolaro et al., 1977; Stewart et al., 1978; Shook et al., 1987). Other gastrointestinal effects of opioids, as described below, may also be mediated by either central or peripheral chemosensitive sites.

Recognition of multiple types of opioid receptors in the central nervous system and in the periphery (Martin et al., 1976; Lord et al., 1977) has provided new insight into opioid regulatory functions. Availability in recent years of agonist and antagonist ligands with selectivity for the individual types of opioid receptors has provided an opportunity to determine which supraspinal, spinal and peripheral receptors are responsible for opioid analgesic effects and for opioid gastrointestinal effects. Selective agonists have been given by intracerebroventricular (i.c.v.) and intrathecal (i.t.) injection techniques to ensure that agonists reach critical sites or to limit their actions to specific sites. The major selective agonists employed were DAMGO ([D-Ala^2, N-methyl-Phe^4, Gly^5-ol]enkephalin) and PL017 ([MePhe^3, D-Pro^4]morphiceptin) for mu receptors (Handa et al., 1981; Chang et al., 1983), DPDPE (cyclic [D-Pen^2, D-Pen^5]enkephalin) for delta receptors (Mosberg et al., 1983), and U-50,488 (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate) for kappa opioid receptors (Von

Voigtlander et al., 1983). In most experiments, morphine has been employed as a standard, mu-preferring opioid agonists.

Analgesia

The antinociceptive (analgesic) effects of DAMGO, DPDPE, U-50,488 and morphine have been evaluated in unanesthetized rats and mice. In rats, all four agonists produced dose-related analgesia after i.c.v. administration in the hot plate test (Galligan et al., 1984). The order of potency was DAMGO > morphine > DPDPE > U-50,488. This study established that a highly selective delta opioid agonists, DPDPE, can produce analgesia after i.c.v. administration.

More extensive studies of analgesic actions of receptor-selective opioid agonists have been carried out in mice. DAMGO, morphine, DPDPE and U-50,488 produced anaglesia in the mouse writhing test (Porreca et al., 1987). The rank order of potency after i.c.v. administration was DAMGO > morphine > DPDPE > U-50,488. Similar results were found after i.t. administration, except that all agonists were much more active in the writhing test after i.t. than after i.c.v. administration. In the mouse hot plate test, DPDPE was more potent when give i.t. than i.c.v., morphine was approximately equipotent by the two routes, and DAMGO and U-50,488 were more potent when given i.c.v than i.t.

Evidence to date indicates that mu, delta and kappa opioid receptors in the central nervous system can independently mediate analgesia. For example, the analgesic actions of DAMGO, but not DPDPE, are blocked by the mu-selective antagonist, ß-funaltrexamine, where as anaglesic responses to DPDPE, but not those to DAMGO, were blocked by the delta-selective antagonist, ICI-174,864, (Hey-mans et al., 1988).

Morphine, PL017 and U-50,488, but not DPDPE, produced significant analgesia when administered subcutaneously (s.c.) in mice (Shook et al., 1987).

Dynorphin-(1-13) given i.c.v. to mice failed to produce analgesia and attenuated analgesia induced by morphine (Friedman et al., 1981). However, dynorphin was found subsequantly to produce analgesia after i.t. administration to mice or rats (Piercy et al., 1982; Han and Xie, 1982). We found that dynorphin-(l-9) given i.t. in mice produced dose-related analgesia in the warm water tail flick test (Porreca et al., 1983).

Gastrointestinal Transit

Opioids of the morphine type have long been known to inhibit flow of contents through the gastrointestinal tract. Morphine was shown to produce dose-related inhibition of small intestinal transit in rats after i.c.v., s.c. or intragastric administration, with the i.c.v. route approximately 100-fold more potent than the s.c. route (Galligan and Burks, 1983). It was subsequently discovered that morphine can also inhibit gastrointestinal transit by means of actions in the spinal cord of the mouse or rat (Porreca and Burks, 1983; Koslo et al., 1985). The important site(s) of morphine anti-transit actions after systemic administration have been the subject of debate, with advocates for purely peripheral sites of action (Tavani et al., 1979; Manara et al., 1986) and those proposing both central and peripheral sites of antitransit actions (Parolaro et al., 1977; Stewart et al., 1978; Burleigh et al., 1981). In studies using the highly selective mu opioid antagonist, CTP (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2), it was found that i.c.v. administered CTP blocked the analgesia induced by s.c. administered morphine, but not morphine's antitransit effect (Shook et al., 1987). When the peptide antagonist, which does not effectively cross the blood-brain barrier, was given s.c., it blocked the antitransit effects of low (0.3 mg/kg) doses of S.C. morphine, but only partially inhibited the antitransit effects of high (3 mg/kg) doses of s.c. morphine. Thus, with systemic doses that produce analgesia, morphine appears to exert small intestinal antitransit effects by actions both in the periphery and in the central nervous system.

The roles of brain and spinal opioid receptors in mediation of antitransit effects where explored in mice and rats with receptor selective agonists. In mice, DAMGO and morphine given i.c.v. and i.t. produced dose-related inhibition of small intestinal transit (Porreca et al., 1984). DPDPE and U-50,488 were essentially inactive when given i.c.v. However, DPDPE produced dose-related inhibition of transit when administered i.t. U-50,488 produced only modest antitransit effects when given i.t. Similar results were obtained in rats (Galligan et al., 1984). Morphine and PL017 given peripherally (s.c.) produced dose-related inhibition of small intestinal transit in mice (Shook et al., 1987). DPDPE and U-50,488 did not exhibit antitransit effects when administered peripherally.

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Antidiarrheal Effects

Pathological diarrhea results usually from excessive secretion of fluid and electrolytes into the lumen of the intestine. The rate of fluid secretion exceeds the rate of absorption, there is net accumulation of fluid in the lumen, and diarrhea results (Cooke, 1989). Mucosal transport in the small intestine is regulated primarily by neural mechanisms (Cooke, 1989; Sheldon et al., 1989). Brown and Miller (1983) discovered that mucosal transport in the intestine can be regulated by the central nervous system. We examined in mice the antidiarrheal properties of receptor selective opioid agonists. When give i.c.v., morphine, PL017 and DPDPE inhibited experimental diarrhea in a dose-related fashion (Shook et al. 1989). Interestingly, the mu agonists, morphine and PL017 given i.c.v., inhibited diarrhea at doses much lower than those needed to produce analgesia or to inhibit gastrointestinal transit. DPDPE was equipotent in inhibiting diarrhea and in producing analgesia, but did not affect the rate of transit. U-50,488, given i.c.v., inhibited diarrhea only at extremely high doses and had no effect on transit. When given peripherally (s.c.), morphine, PL017, DPDPE and U-50,488 all inhibited diarrhea in a dose-related manner. The antidiarrheal effects of the mu, delta and kappa opioids were probably attributable to effects on mucosal transport of fluid and electrolytes because the antidiarrheal effects occurred at doses lower than those necessary to inhibit gastrointestinal transit.

Gastric Secretion

The effects of receptor-selective opioid agonists on gastric acid secretion were assessed in pylorus-ligated rats (Fox and Burks, 1988). The mu opioids, morphine, PL017, and DAMGO, all produced dose-related inhibition of gastric secretion after i.c.v. injection. When given i.v., larger doses of morphine and PL017 inhibited gastric secretion, but DAMGO had no effect. DPDPE did not alter gastric secretion when given either i.v. or i.c.v. However, i.v. administration of U-50,488 produced a dramatic increase in volume of gastric secretion and acid output, whereas i.c.v. administration of U-50,488 had no effect. These data indicate that mu opioids act in the central nervous system to decrease acid secretion, U-50,488 acts peripherally to increase secretion, and DPDPE has no effect either centrally or peripherally.

Conclusions

Supraspinal and spinal opioid receptors clearly mediate antinociceptive responses in rats and mice. Mu and delta opioid receptors and, to a lesser extent, kappa opioid receptors, are associated with antinociception. Supraspinal, spinal and peripheral mu opioid receptors also mediate gastrointestinal antitransit effects in rats and mice. Spinally administered DPDPE and U-50,488 produced modest antitransit effects, but essentially no effects after i.c.v. or s.c. administration. In contrast, mu, delta and kappa agonists can act at both brain and peripheral opioid receptors to produce antidiarrheal effects. Qualitatively different effects associated with different opioid receptors were observed with gastric acid secretion.. Mu opioids appear to act in the brain to inhibit gastric secretion, U-50,488 acts peripherally to increase secretion, and DPDPE had no effect.

It is evident that opioids can act at multiple types of receptors in the brain, spinal cord and periphery to produce functional changes. The specific changes observed depend on the site of opioid action, the types of receptor involved, and the specific endpoint measured.

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Acknowledgements

The research described in this report was supported by USPHS grants DA02163, DK33547, and DK36289.

AUTHOR:

Thomas F. Burks, Ph.D., Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724

Opioid Delta Receptor Involvement in Behavioral and Neural Plasticity

Joe L. Martinez, Jr., Gery Schulteis, Brian E. Derrick, Susan B. Weinberger, Teresa A. Patterson, Edward L. Bennett and Mark R. Rosenzweig

Opioid peptides affect complex cognitive furctionirq, as assessed in a variety of conditioniq paradigm, in both rodents and monkeys. learning and memory of both avoidance and appetitive conditioning tasks are influenced by opioids (see Schulteis et. al., 1989, for review). In many instances the behavioral actions of opioid peptides depend on opioid receptor activation, since many of their effects are blocked by opioid antagonists (see Martinez et al., 1988, Schulteis et. al., 1989, for review). Because all of the naturally occurring opioid peptides have at least some affinity for all types of opioid receptors (Kosterlitz and Paterson 1985), it is not yet known which opioid receptor type(s) contributes to the behavioral effects observed following treatment with opioid peptides. The recent development of selective opioid receptor agonists and antagonists (see Schulteis et al., 1988, Schulteis and Martinez 1989, for references) has allowed the commencement of the analysis of the contribution of specific opioid receptor types to particular behavioral actions. This paper focuses on the role played by δ opioid receptors in three different situations: l) avoidance conditioning in rats and mice, 2) taste avoidance conditioning in chicks, and 3) long-term potentiation in the rat hippocampus.

AVOIDANCE CONDITIONING IN RODENTS AND CHICKS

Our studies investigate the modulatory role of the opioid pentapeptide [leu]enkephalin (LE) on learning and memory (see Martinez et. al., 1988, Schulteis et. al., 1989, for review). LE has higher selectivity for the δ opioid receptor than any other naturally occurring opioid peptide; it has only moderate affinity for the n receptor and negligible affinity for κ receptors (Kosterlitz and Paterson 1985). [Met]enkephalin (ME), which shares many of LE's effects on conditioning, has a slightly greater affinity for μ receptors than LE, but it also possesses responsible selectivity for δ receptors. Thus, we hypothesized that the effects of enkephalins on the acquisition and retention of learned

behaviors are mediated by δ opioid receptors, and that this type of opioid receptor plays an important role in modulating the strength of a learned response.

An initial experiment testing this hypothesis used the selective δ receptor agonist [D-pen$_2$,D-pen^5]enkephalin (DPDPE) (Schulteis et al., 1988). In this study, male Swiss-Webster mice were given intraperitoneal (i.p.) injections of LE or DPDPE, 2 min prior to training in a one-way active avoidance paradigm. Both IE (30 and 100 µg/kg) and DPDPE (11.6 µg/kg) impaired acquisition of the avoidance response. The dose-response functions for both peptides were U-shaped, an effect commonly seen in studies of drug effects on learning and memory (see Schulteis et al., 1989, for further discussion). If animals are retested 24 hrs after training, then those animals that were impaired during acquisition were still impaired upon retest (Schulteis et. al., 1988). Thus injections of LE or DPDPE prior to training resulted in learning impairments; the peptides did not simply impair the ability of the animals to perform the response during training.

Since stimulation of δ receptors by exogenously administered ligands impairs learning, we hypothesized that the effects of these ligands would be reversed by concurrent administration of a selective δ antagonist, and that blockade of δ receptors with an antagonist which prevents endogenous opioids with affinity and selectivity for δ receptors from binding to these sites, would enhance learning. The results of several experiments support these hypotheses. Concurrent i.p. injection of LE (100 µg/kg) and ICI 174,864 (1.0 mg/kg), a selective δ receptor antagonist, results in complete reversal of the impairment in learning produced by LE (Schulteis and Martinez 1989). In addition, both ICI 174,864 (3.0 mg/kg) and the less potent, but equally selective analog ICI 154,129 (30 and 100 mg/kg), by themselves significantly enhance acquisition of the active avoidance response (Schulteis and Martinez 1989, Schulteis et. al., 1988). Thus endogenous opioid peptide systems may modulate learning by acting through the δ opioid receptor. We suggest that LE and perhaps the closely related peptide ME are the endogenous ligands involved in this effect.

Studies in rats using an automated shelf-jump avoidance paradigm confirmed our results in mice. Both LE (1.0 and 3.0 µg/kg) and DPDPE (1.16 but not 11.6 µg/kg) were found to impair acquisition of the shelf-jump response in rats (Weinberger et al. 1988). A similar level of impairment was produced by equimolar doses of the two peptides, and the dose-response function for the impairment produced by DPDPE in the automated shelf-jump task was U-shaped. These results, together with our findings that DPDPE and LE both impair acquisition of a one-way active avoidance response in mice, and that LE impairs acquisition of the same response in

rats, further support our suggestion that δ opioid receptors are implicated in the effects of LE on conditioning. In addition, these results indicate that the involvement of δ receptors in acquisition impairment extends to at least two rodent species and to two different avoidance conditioning tasks.

Recently we investigated the generality of the involvement of δ opioid receptors by studying their role in taste avoidance conditioning in the two-day old chick (Patterson et. al., 1989). Chicks naturally peck at a bright shiny bead, and readily learn to avoid pecking if the bead is coated in a bitter-tasting substance. This single-trial learned response is quite robust, since 80-90% of saline-treated control animals will avoid the bead when it is represented 24 hrs after the original training.

LE (0.01 - 10.0 nmoles/hemisphere) and [D-pen$_2$,L-pen^5]-enkephalin (DPLPE; 0.003 - 0.10 nmoles/ hemisphere) were administered to chicks intracranially (i.e.) into the medial hyperstriatum ventrale (MHV) 5 min before training. Both drugs produced U-shaped dose-response functions, with a 1.0 nmole dose of LE and a 0.03 nmole dose of DPLPE impairing acquisition of the response. We also found that ICI 174,864 (10.0 nmoles/hemisphere) , when given in combination with the impairing dose of LE or DPLPE, reversed the effect of both peptides. These latter studies provide further evidence that the effects of LE and DPLPE on learning in the chick are mediated by δ opioid receptors.

The effects of δ selective agonists and antagonists on acquisition of the taste avoidance response following i.e. injection in chicks are remarkably similar to those described above for rodents. Selective δ opioid receptor agonists impair conditioning, produce U-shaped dose-response functions, and are antagonized by δ selective antagonists in both rodents and chicks.

LONG-TERM POTENTIATION IN THE CA3 REGION OF THE RAT HIPPOCAMPUS.

In addition to the modulatory effects on avoidance conditioning produced by δ receptor agonists and antagonists, we are investigating a long-lasting form of synaptic plasticity (long-term potentiation: LTP) in the rat hippocampus that may be initiated by activation of δ receptors.

UTP is an enduring change in synaptic efficacy observed following high frequency activation of afferent projections to and within the hippocampal formation (see Bliss and Lynch 1988 , for review). LIP is a promising model for the synaptic plasticity that may underlie some types of memory. LTP induction in many hippocampal pathways requires activation of the N-methyl-D-aspartate (NMDA) receptor, a glutamate

receptor subtype requiring both glutamate and sustained postsynaptic depolarization for its activation. The mossy fiber projection to area CA3, a putative enkephalinergic pathway, is unique in that NMDA receptor antagonists do not block LTP at messy fiber-CA3 synapses (Harris and Cotman 1986). By contrast, naloxone, an opioid receptor antagonist, does block LTP induction at these synapses (Derrick et. al., submitted, Martin 1983). This blockade is not observed with (+)-naloxone, an inactive stereoisomer, indicating that the effects of naloxone on LTP are due to antagonism of opioid receptors. Induction of LTP in responses evoked in commissural afferents to the same population of CA3 pyramidal cells is unaffected by naloxone (Derrick et. al., submitted).

Exogenous application of agonists selective for μ or δ receptors produces marked excitation of hippocampal pyramidal cells (see Corrigal 1983, for review). Although it was suggested that GABAergic inhibition may be indirectly involved in this effect (see Bliss and Lynch 1988, for review) and that the blockade of LTP induction by naloxone may result from a blockade of these disinhibitory effects of endogenous opioid peptides (Bramham et. al.. 1988, Martin 1983), we found that coadministration of bicuculline, a GABA antagonist, and naloxone did not overcome the naloxone blockade of LTP. Together these findings suggest that LTP induction at the mossy fiber-CA3 synapse uses a unique mechanism that directly involves opioid receptor activation (Derrick et. al., submitted).

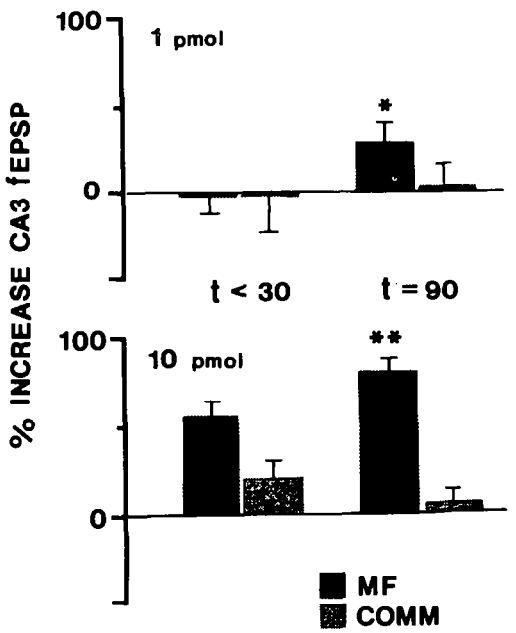

Figure 1. The effect of DPDPE (1.0 and 10 pmoles) on field evoked by stimulation of mossy fiber or commissural afferents. Maximal increases were observed at 0-30 min and at 90 min after DPDPE application. Data are reported as the maximal percent increase from baseline. Mann-Whitney U-test, two-tailed:
* $p<0.05$,
** $p<0.01$.

The high frequency stimulation needed to induce LTP may release opioid peptides (Bramham et. al., 1988). This hypothesis suggests that exogenous application of opioid agonists should mimic the effects of high frequency stimulation of enkephalinergic pathways. We found that DPDPE produced increases in mossy fiber-CA3 evoked responses that were significantly greater than those observed in the non-opioid commissural pathway to CA3 (Derrick and Martinez 1989), and that DPDPE produced a long lasting enhancement in mossy fiber-CA3 responses qualitatively similar to LTP. The increase in mossy fiber responses was greater 90 min following than immediately following peptide application (see Figure 1). By contrast, DPDPE effects on commissural responses had nearly returned to baseline by 90 min. Similar effects were not observed following application of agonists selective for μ or κ receptors. Together, these results suggest that induction of LTP at mossy fiber-CA3 synapses is dependent on opioid receptor activation, and that δ opioid receptors may be the implicated receptor type.

CONCLUSIONS

All of the studies reviewed above suggest that δ opioid receptors play an important role in behavioral and neural plasticity. The modulatory actions of enkephalins in several tasks and species (mice, rats, chicks) appear to be mediated by stimulation of the δ receptor. Further S receptors may play a direct role in the induction of LTP at mossy fiber-CA3 synapses. Thus, it is evident that δ receptors appear to be critically involved in mechanisms of neural plasticity at several levels of analysis.

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ACKNOWLEDGEMENTS

Supported by PHS grants DA04195 (JIM) and DA04795 (MRR, JIM, ELB) from NIDA, and NRSA fellowships DA05334 (GS), DA05374 (BED), and DA05313 (SBW).

AUTHORS

Joe L. Martinez, Jr., Ph.D.
Gery Schulteis
Brain E. Derrick
Susan B. Weinberger, Ph.D.
Teresa A. Patterson, Ph.D.
Edward L. Bennett, Ph.D.
Mark R. Rosenzweig, Ph.D.

Department of Psychology
University of California, Berkeley, CA 94720

Thermoregulation and the Opioid System

Martin W. Adler and Ellen B. Geller

One of the most commonly utilized measures of the actions of a variety of drugs from numerous pharmacological classes is body temperahure (T_b). Despite that, little thought is given to the relationship between T_b, and thermoregulation. T_b, is merely a reflection of heat-gain and heat-loss mechanisms:

Body Temperature = Heat Gain + Heat Loss

Just because a drug alters T_b does not mean that it interferes with or affects thermoregulation. It does not mean that it alters the ability of the body to maintain a temperature around a given point, the set point. In mammals, including the human, T_b is regulated in an exquisitely narrow range. This tight regulation allows an organism to maintain a thermodynamically stable internal environment in which the rates of a myriad of biochemical reactions can be altered without having to compensate for changes in ambient temperature. We become aware of thermoregulation only when something like infection occurs with resulting fever. If the temperature keeps going up as the result of release of substances such as endogenous pyrogen or IL- 1 or as a result of malignant hyperthermia, we begin to look for ways to return the T_b to normal On the other hand, we look to raise the T_b in circumstances where hypothermia occurs, as with stranded winter mountain climbers, inadequate ambient temperature in the homes of the aged, or a child falling through the ice and suffering from the so-called “near-drowning” syndrome. Furthermore, physicians are always looking for better ways to alter T_b and exert thermoregulatory control when they cool patients during cardiac surgery or heat patients in treating some forms of cancer. Our crude ability to modify and modulate the thermoregulatory system is impeded by our poor understanding of the system itself. If we knew more about it, perhaps we could more effectively use it in conditions such as cardiac surgery, preservation of organs for transplant, treatment of stroke, treatment of trauma to the spinal cord, and treatment of malignancies.

There appear to be three components in temperature regulation: a set of sensors to monitor ambient and core temperatures, portions of the CNS (especially the hypothalamus) to receive and coordinate this information, and the effector system of nerves and organs utilized by the regulator to make appropriate responses. Although the sensory and effector systems are fairly well understood, we know very little about the regulator (coordinator) part of the system. The recent data that has been forthcoming about the opioid system indicates that this system is intimately involved in thermoregulation and, perhaps, is the coordinating or

regulatory part of the system. Before giving some indication of what we believe that involvement to be, it may be worthwhile summarizing the very confusing literature about the possible role of several neurotransmitters in thermoregulation.

Among the most frequently implicated transmitters in thermoregulation are norepinephrine, dopamine, serotonin, acetylcholine, and histamine (Cox and Lomax, 1977; Myers, 1980; Lomax and Schönbaum, 1979). Although it is generally accepted that all of these substances play some role, the degree of their involvement is still very much unresolved. The only neurotransmitter that virtually all investigators agree is involved in therrnoregulation is norepinephrine (NE). When microinjected into the preoptic anterior hypothalamus (POAH), NE causes a dose-dependent hypothermia in most species studied. This effect can be blocked by prior injection of an alpha adrenergic antagonist into the hypothalamus (Rudy and Wolf, 1971). Dopamine (DA) has also been shown to induce hypothermia when injected into the POAH in most species, and this decrease can be antagonized by DA receptor blockers. Based on this and other evidence, it has been proposed that catecholamines mediate heat loss or block heat production (Myers, 1980). Other investigators have reported opposite results in rats, however (Cox et al., 1980; Lin et al., 1982). Likewise, there is contradictory evidence for the role of serotonin (5-HT). In most species, 5-HT elicits a dose-dependent hyperthermia when microinjected into the POAH and is thought to be involved in heat-gain mechanisms (Myers, 1974; Borsook et al., 1977). However, elevating levels of 5-HT in hypothalamus by stimulation of the midbrain raphe, or by administration of the precursor 5-HTP, or by inhibiting 5-HT uptake has been shown by others to cause a decrease in metabolism, cutaneous vasodilation, and hypothermia in rats (Lin, 1984). The story on acetylcholine (ACh) is even more confusing. Whether it results in a hyperthemtic or hypothermic effect is very much dependent on the species and route or brain site of administration. It has been implicated in both heat-production and heat-loss pathways (Lin, 1984; Myers, 1980). Results with histamine have been more consistent: it causes hypothermia at normal ambients whether administered systemically or centrally (Lomax and Green, 1979). Of course, there are a host of possible reasons as to why after so many years of research we still have no clear picture of the role these and other transmitters play in regulating T_b, but it appears that this system relies on redundant pathways and balancing complex interrelationships among neurotransmitters, neuropeptides. hormones, and cations in order to maintain its remarkable stability. The opioid peptides, at a minimum, may just represent one more group of substances that can affect T_b. On the other hand they may turn out to have a command function in the overall system.

We can begin the opioid story with a brief review of the effects of these drugs on T_b. Until the last few years, we knew little about the actions of opioids other than morphine. What was known was that the effect of morphine given by a parenteral route varied with the species.

TABLE 1 Morphine on Body Temperature in Various Species

HYPOTHERMIA	HYPERTHERMIA	DUAL EFFECT
dog	cat	rat
rabbit	horse	mouse
bird	cow	primate
	goat	

A further confounding factor is that the effect is often determined by the route of administration. For example, in the rat, morphine has a well-known hyperthermic effect when admininistered by a parenteral route in low doses and a hypothermic response in high doses. On the other hand, intracerebroventricular (icv) administration results in only a dose-related hyperthermia (Geller et al., 1986)

Studies in our laboratory have been primarily in the young adult male Sprague-Dawley rat. T_b is measured in most instances by means of a temperature probe inserted 7 cm into the rectum. Animals are essentially unrestrained and T_b is measured several times over a 3- to 4- hour period with each animal acting as his own control (Geller et al., 1986). Unless stated otherwise, the effects of all the opioids and opioid peptides on T_b are blocked by naloxone. After testing a large number of opioids administered by the subcutaneous (SC) and the icv route, we found that the drugs separated into 5 classes based on the nature of the effects they produced, as described below.

TABLE 2 Effects of Opioids on Body Temperature in Rats

GROUP 1: dual response; antagonized by naloxone; stereospecificity
GROUP 2: hyperthermia; antagonized by naloxone; stereospecificity
GROUP 3: hypothermia; partially antagonized by naloxone; stereospecificity
GROUP 4: little effect; naloxone causes hypothermia
GROUP 5: little or no effect

Our first attempt to put some order into the chaos was a 2-receptor model (Geller et al., 1983.) This dual receptor hypothesis might explain the dual effect of morphine on T_b,. Briefly, the low-dose hyperthermia is due to the presence of a high number of receptors with high affinity but low intrinsic activity. The high-dose hypothermia is due to a smaller number of hypothetic receptors with lower affinity but high intrinsic activity. As we continued our work, we began to define the components of that system and came up with our current hypothesis that the thermal actions of the opioid system are based on a 2-receptor model. There is a p-receptor-mediated hyperthermia and a K-receptor-mediated hypothermia. Further, the μ actions are, at least in part, due to actions in the brain, while κ actions are primarily outside the brain (Geller et al., 1986). We were able to predict that a drug such as U-50,488H, the highly selective κ agonist, would cause a drop in T_b and it did (Adler et al., 1986). Since the guinea pig has a preponderance of κ receptors, we predicted that it would be even more sensitive than the rat to the hypothermic effects of U50,488. As expected, it was (Adler et al., 1988).

Using gradient-layer calorimeters, we have begun to look at whether heat-gain or heat-loss factors are responsible for the changes in T_b with μ and κ agonists in unrestrained animals. The precipitating event for the drop in T_b, with U50,488 appears to be a decrease in oxygen consumption. Decreased heat loss probably results from a homeostatic attempt to conserve heat and a decrease in available heat with the decline in VO_2. If a drop in T_b is always associated with κ activity, then we would expect similar patterns regardless of the opioid inducing the effect. Methadone, when given in doses high enough to produce hypothermia, causes similar changes in VO_2 and Q. The only difference lies in the recovery from the hypothermia, which may be attributed to methadone's actions on μ receptors (Lynch et al., 1987).

To further explore the hypothermic effect of κ receptor activation, we looked at the combination of U50,488 with another drug known to drop T_b. We chose chlorpromazine (CPZ). In this case, the hypothermia results primarily from an increased heat loss, rather than an effect on O_2 consumption. If we combine U50,488 and CPZ, we get a composite of the effects seen with the individual drugs, as illustrated in figure 1.

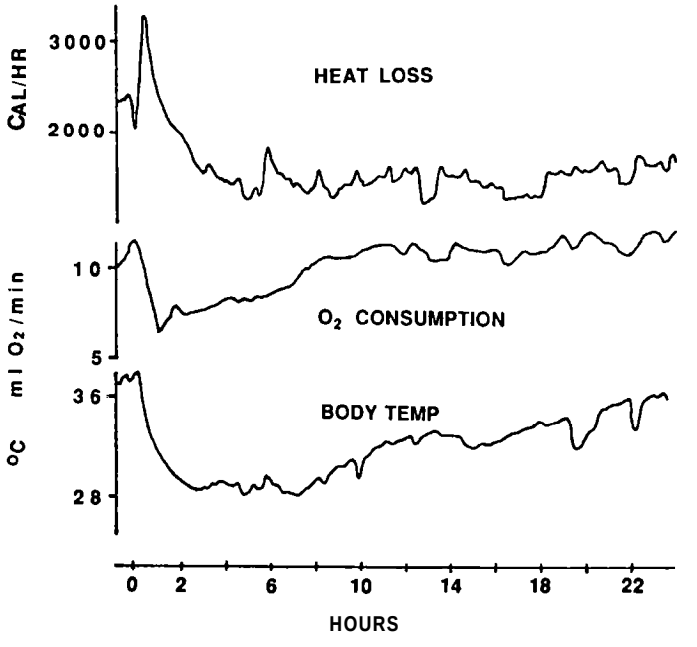

FIGURE 1 Potentiation of U50,488 and CPZ. At time 0, rat received SC injections of 5 mg/kg CPZ and 80 mg/kg U50,488H, dissolved in saline.

The increased heat loss caused by CPZ added to the decrease in O_2 consumption caused by U50 results in a potentiation of the hypothermia. At the doses used, each drug alone lowered T_b by about 2°C. Combined, however, the temperature dropped by over 8°C. Individual animals have shown drops of over 11°C (Adler and Geller, 1987). There are no deaths at normal ambient temperature. The reason for the huge drop in T_b is a large spike in heat loss plus a decrease in VO_2.

There is certainly no question that calorimetry can provide important insights into the mechanisms by which opioids affect thermoregulation. A number of other approaches are also needed. For example, changing the ambient temperature can tell us something about set point. Another approach is behavioral thermoregulation. If an animal's physical ability to choose an ambient temperature is not impaired, we can learn much about set point by determining if an animal chooses a warmer or cooler environment. Such a technique has been used to study the effects of μ. κ, and 6 agonists in rats (Spencer et al., in press.)

More recently, we have begun to use selective opioid peptides and opioid antagonists to pick apart the effects of opioids on T_b and thermoregulation. Although these studies are still in their initial phase, there are a few findings of special interest. ID-Ala[2], N-MePhe[4], Gly[5]-ol]-enkephalin (DAGO), a μ-selective opioid peptide, elicits a dose-related hyperthermia preceded by a small, transient. hypothermia. We felt that this hypothermic effect might be the result of some activity at κ receptors and, indeed, the κ-selective antagonist nor-BNI (Portoghese et al., 1987) antagonized this initial decrease in T_b. Interestingly, the purportedly k-selective antagonist, ß-FNA, only partially prevented the DAGO-induced increase in temperature when given 18-24 h before DAGO. It also only partially blocked the hyperthermia caused by icv morphine.

Surprisingly, both the low-dose hyperthermia and the high-dose hypothermia produced by SC morphine could be blocked by naloxone or either of the more selective antagonists. We had not expected these results based on our model. (In fact, the less selective κ antagonist WIN 44441 had blocked the high but not the low dose effect of morphine.) Just as surprising was the finding that neither antagonist blocked the hypothermia caused by the purported κ agonist dynorphin. Naloxone, even at 10 mg/kg, did not completely block it, either. On the other hand, as would be predicted from our model, the hypothermia produced by the K-selective U-50488H was antagonized by nor-BNI and naloxone but not by ß-FNA. Although additional doses and routes of administration need to be done, these preliminary results may indicate that the model needs to be modified to accommodate subtypes of μ and κ receptors or an as yet unknown interaction between the receptors. In our hands, DPDPE, the highly selective 6 agonist, and naltrindole. the selective 6 antagonist, appear to have no significant effect on T_b either individually or in combination.

We have focused on μ and κ because the evidence is strong, not only from our data but from studies in several other laboratories, that μ is a receptor mediating primarily hyperthermic responses while κ is predominantly involved in mediating hypothermic effects. Although the activation of 6 receptors may produce some small effects on T_b, them is no compelling evidence that it has a significant role in thermoregulation. The same is true for σ receptors in the rat. Perhaps the use of calorimetry, along with behavioral thermoregulatory methods and studies at various ambient temperatures, will shed more light on the question of the role of opioids in thermoregulation. It is hoped that such studies will provide us with the information needed for important contributions to therapeutics as well as increase our knowledge about the functions of the endogenous opioid system.

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ACKNOWLEDGEMENTS

This work was supported in part by grant DA00376 from NIDA. U50,488H was a gift of The Upjohn Company.

AUTHORS

Martin W. Adler, Ph.D. and Ellen B. Geller, M.A., Department of Pharmacology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140.

Interactions of the Opioid and Immune Systems

Robert M. Donahoe

The discipline of psychoimmunology is concerned with the inter-dependencies between neural and immune processes. The conceptual premise of this discipline is that immunological function can be influenced by neuroendocrine and behavioral processes and vice versa. Since addicting drugs of abuse modulate both neural and immune functions, these substances make interesting probes for study of the neuroimmune network. Indeed, the foundations of psychoimmunology can be traced to observations made over seventy years ago that morphine modulates phagocytic capacity of human leukocytes (Archard et al., 1909).

Discoveries in the early 1970s (Pert and Snyder, 1973; Simon et al.. 1973; Terenius, 1973) that opiates exert their effects through specific binding sites (receptors) on the cell surface and that such receptors are the basis for intersystemic responses to both exogenous and endogenous opioids (Hughes et al., 1975) set the stage to appropriately conceptualize the neuroimmune effects of opiates. There was a growing awareness at the time that opiate addicts frequently experience elevated levels of opportunistic infections and cancer (Sapria, 1968; Harris and Garret, 1972; Louria, 1974). Evidence was also accumulating that addicts experience clinically detectable signs of immune depression (Brown et al., 1974). These circumstances led to consideration of the hypothesis that opiates may directly influence immune function. It was not recognized then, and is still evident today that this is not an easily testable hypothesis. The role of opiates themselves in induction of immunodeficiency cannot be tested prospectively in humans and retrospective studies suffer from the uncertainty that immune depression evident in addicts may be the result of immune paralysis induced by frequent exposure to multiple foreign antigenic insults and repeated infections rather than a direct effect of opiates. Notably, the findings of Falek et al., (1972) were of particular relevance to the uncertainties inherent to this situation.

In cytogenetic analyses using phytohemmaglutinin-stimilated whole blood cultures, Falek et al. (1972) showed that heroin addicts had elevated - levels of chromosomal damage. Since their analyses were dependent on errant mitotic events during DNA replication, they demonstrated a deficit in lymphocytes that was not easily attributable to immune paralysis. In a separate study (Madden et al., 1979), they showed that this damage was not likely to be related to direct chelation of DNA by opiates but rather to inhibition of DNA repair mechanisms which further suggested some direct metabolic effect of opiate binding to T-cells. Thus, their studies encouraged further consideration of the notion that opiates might influence T-cells directly.

Their cytogenetic observations led the Falek group to examine the immune status of heroin addicts using the total T-cell E-rosette assay as an immunological measure of T-cell functional integrity. This procedure is a receptor-mediated assay which exploits the ability of T-cells to combine specifically with sheep erythrocytes (E) in a microscopically visible rosette formation. Using this assay, McDonough, et al. (1980) showed that heroin-addict lymphocytes exhibited a naloxone-reversible depression of E-rosette formation which suggested that opiate receptors on the lymphocytes were responsible for the effect observed. Contemporaneous with these findings, Wybran et al. (1979) showed that morphine used in vitro also caused depression of E-rosette formation through interaction with naloxone-reversible stereospecific opioid receptor sites on the T-cell. Taken together, these studies confirmed the suspicion that the immunological effects of opiates could be mediated directly on T-cells within the immune system.

Several pharmacological studies have since identified opiate-binding sites on lymphocytes, including sites for naloxone on purified T-cells (Madden et al., 1987). Others have shown that morphine compromises the immune status of mice relative to their ability to reject bacterial infections (Tubaro et al., 1983). Also, there is considerable literature documenting the fact that many different branches of the immune armamentarium are affected by alkaloid and peptide opioids (reviewed by Donahoe and Falek, 1988). These findings and the other evidence linking opiate use with immunomodulatory phenomena as discussed previously provide strong support for the hypothesis that opiate and other drug abuse are direct causal factors in opportunistic diseases like AIDS. However, as with all suspicions of the potential for opiates to be immunocompromising, this hypothesis, as applied to AIDS, is a difficult one to prove.

The main questions are how to distinguish immunological effects of opiates from those attributable to other milieu factors common in addiction and how to assess immunocompetency prospectively? The nature of the former

issue has been discussed above. The latter question is basic to all clinical *in vitro* evidence that immunoresponsiveness of an individual is depressed cannot be taken as a priori proof that that individual is immunocompromised. Such proof can only be obtained de facto, i.e., when it is apparent that the individual in question is incapable of warding off opportunistic infections and cancers. therefore, with addicts, there is an inextricable linkage of one research problem with the other which makes study of the issues involved very complex, that is, evidence of immunocompromised conditions cannot be attributed to the opiate per se and evidence that opiates can depress immune parameters that are measurable in *in vitro* assays cannot be taken as unequivocal proof of immunocompromised immune status.

Because of these circumstances, research strategies to assess the immunological impact of opiates and other drugs of abuse must be based on development of strong circumstantial evidence. In several previous reviews (Donahoe, 1988; Donahoe and Falek, 1988), we have presented the conceptualization that the immunocompromising potential of opiates and other addicting drugs of abuse is best estimated by thoroughly analyzing the interconnections between the neuroendocrine and immunological systems while using the drugs of interest as pharmacological probes. In essence, this implies that understanding the way drugs affects immunity requires knowledge of the direct effects of such drugs on the cells of the immune system, their indirect effects which occur through induction of neuroendocrine products with immunoregulatory properties and, perhaps also, modulation of peripheral synapses between neurons and immune cells, as well as the combination of all of these potential types of interactions.

Our current in vitro investigations stem from this conceptualization and have focused on the ability of opiates and other drugs of abuse to modulate expression of T-cell surface receptors that are pertinent to the regulation of immunoresponsiveness. This direction was chosen as the result of previous studies (Donahoe *et al.,* 1985; Donahoe *et al..* 1988) that identified receptor modulation as a major, and probably key means by which drugs of abuse influence immune function. In this context, modulation of immunity by drugs of abuse is the consequence of altered expression of surface receptors on cells of the immune system regardless of whether the effect is mediated directly by the drug of interest or indirectly through alteration in neuroendocrine secretions. For this reason, we are trying to understand how drugs of abuse and the neuroendocrine changes they induce affect expression of several different regulatory molecules on T-cells which we know from other studies (Donahoe *et al.,* 1985; Donahoe *et al.,* 1986; Donahoe and Falek, 1988) to be affected by drug exposure *in vivo* and *in vitro.*

Principally, we have been analyzing drug effects on CD2, CD4 and CDS antigenic markers on T-lymphocytes. We also plan to include the T-cell receptor/CD3 complex and class I and II histocompatibility markers as well as the other molecules pertinent to T-cell function. By understanding how opiates and other drugs of abuse influence both independent and interdependent expressions of these T-cell antigenic markers, we should be in a position to better delineate the immunoresponsive effects of these drugs and thereby estimate their ability to compromise immune function as well as assess the means by which such compromise immune comes about. Since the CD4 marker is the viroreceptor for the AIDS virus, HIV1, this approach also bears special relevance to determining how drugs of abuse that modulate this molecule can influence infections with this virus.

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ACKNOWLEDGEMENTS:

Grant support is acknowledged from the following sources:

1) National Institute on Drug Abuse: DA01451; DA04400; DA0498
2) State of Georgia, Department of Human Resources

AFFILIATION:

Department of Psychiatry
laboratory of Psychoimmunology
Emory University School of Medicine
Georgia Mental Health Institute
1256 Briarcliff Road, N.E.
Atlanta, GA 30306

Site-Directed Affinity Ligands as Tools to Study the Phenomenology and Mechanisms of Morphine-Induced Upregulation of Opioid Receptors

Richard B. Rothman, Joseph B. Long, Victor Bykov, Kenner C. Rice and John W. Holaday

Selective site-directed irreversible ligands have proven useful for the in viva and in vitro study of opioid receptor subtypes (Portoghese *et al.* 1979; Goldstein, and James, 1984; Simonds *et al.* 1985; Rothman *et al.* 1988; Holaday *et al.* 1986) several examples of which are described by the other participants of this symposium. In our laboratories, we have attempted to determine the specific targets of various site-directed irreversible ligands. This approach has led to the further delineation of opioid receptor subtypes, which in turn has permitted the selective assay of these various subtypes of opioid receptors. Although a thorough review of the model of the opioid receptors presented in Table 1 is beyond the scope of this paper, in vitro (Rothman *et al.* 1985b; Rothman *et al.* 1985a; Demoliou-Mason, and Barnard, 1986),

Table 1
Effect of Site-Directed Irreversible Ligands on Opioid Receptor Subtypes

Agent	μ_{ncx}	$\mu_{cx} \Leftrightarrow \kappa_{cx} \Leftrightarrow \delta_{cx}$	δ_{ncx}	κ_2	κ_1
BIT[1]	D	D	N	N	N
FNA[2]	N	A	N	PD	N
UPHIT[3]	N	N	N	N	D
FIT[1]	N	A	D	N	N
(+)-TSF[4]	N	A	D	N	N

Tabulated are the effects of the irreversible ligands on opioid receptor subtypes. "D" means the membranes can be depleted of a binding site, "A" means the binding site is altered, "N" means no effect, and "PD" means partially depleted. (+)-TSF refers to (+)-trans-3-methylfentanyl isothiocyanate [1](Rothman *et al.* 1984).[2](Rothman *et al.* 1988).[3](De Costa *et al.* 1989). [4](Kim *et al.* 1988).

and in vivo evidence (Vaught *et al.* 1982; Holaday *et al.* 1986; Heyman *et al.* 1989; Sheehan *et al.* 1986), collectively indicates the existence of an opioid receptor complex composed of interacting μ_{cx}, δ_{cx} and κ_{cx} binding sites (the term "cx"means "in the complex"), as well as μ_{cx}, δ_{cx} and κ sites not associated with the receptor complex (the term ncx means "not in the complex).

As illustrated in Table 1, the judicious use of site-directed irreversible ligands, together with appropriate [^{3}H]ligands, permits selective assay conditions. For example, since [^{3}H][D-ala^2,D-leu^5]enkephalin labels both the δ_{cx} and δ_{ncx} binding sites, pretreatment of membranes with FIT permits selective assay of the δ_{cx} site, while pretreatment of membranes with BIT permits selective assay of the δ_{ncx} site using this radioligand (Rothman *et al.* 1984). Using this approach, we demonstrated that chronic morphine upregulated the δ_{cx} binding site (Rothman *et al.* 1986a). In this paper we review and summarize our studies of the phenomenology and mechanisms of morphine-induced upregulation of opioid receptors.

METHODS

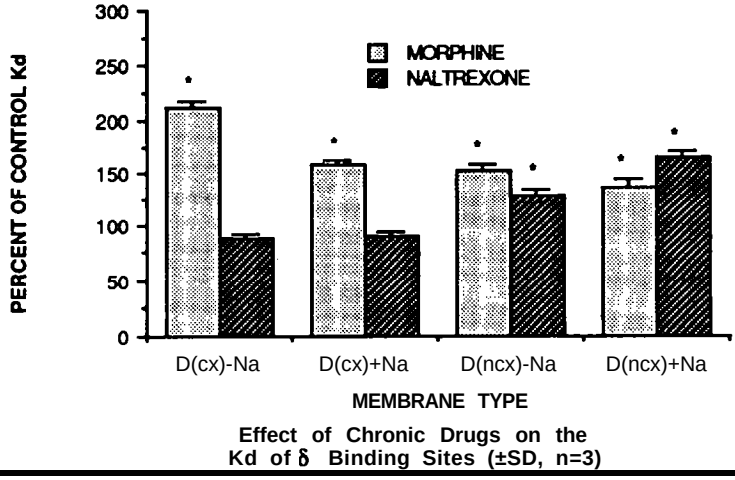
Drug treatments and i.c.v. injections of ß-funaltrexamine are described in detail elsewhere (Danks *et al.* 1988; Rothman *et al.* 1988). Frozen membranes pretreated with BIT or FIT were prepared from whole rat brain (minus cerebellum) as previously described (Danks *et al.* 1988). Briefly, lysed-P2 membranes were divided into two aliquots. The first ("+Na-membranes") were resuspended with 50 mM TRIS-HCL, 0.4 M NaCI, pH 7.4, incubated for 60 min at 25° C, and subsequently washed three times by centrifugation. During this procedure, the second pellet was kept on ice ("-Na-membranes"). The two pellets were then pretreated with either 1 µM BIT or 1 µM FIT as previously described (Rothman *et al.* 1984) and kept frozen at -70° C until assayed. [^{3}H][D-ala^2,D-leu^5]enkephalin assays proceeded for 4 to 6 hr at 25° C in 10 mM TRIS-HCL, containing 100 mM choline chloride, 3 mM $MnCl_2$, 1 mM 2-mercaptoethanol, and a protease inhibitor cocktail. Nonspecific binding was determined using 20 uM levallorphan.

[^{3}H][D-ala^2,D-leu^5]enkephalin binding surfaces were generated by displacing two concentrations of [^{3}H]ligand by concentrations of [D-ala^2,D-leu^5]enkephalin. The binding surfaces were fit to a one site binding model using MLAB (Knott, and Reece, 1972) for the best-fit estimates of the Kd and Bmax. The method of binding surface analysis is described in greater detail elsewhere (Rothman, 1986b; Rothman *et al.* 1988). In the figures, an asterisk (*) indicates significantly different from control (t-test, $p<0.01$).

RESULTS

using -Na-membranes. The δ_{cx} binding sites upregulated by chronic morphine were apparently labile to preincubation in 0.4 M NaCl. Chronic naltrexone increased the Kd of the δ_{ncx} binding site (Figure 3), using both -Na and +Na membranes. In contrast, chronic morphine significantly increased the Kd of both δ binding sites using both -Na- and +Na-membranes.

Figure 3



Effect of Chronic Drugs on the Kd of δ Binding Sites (±SD, n=3)

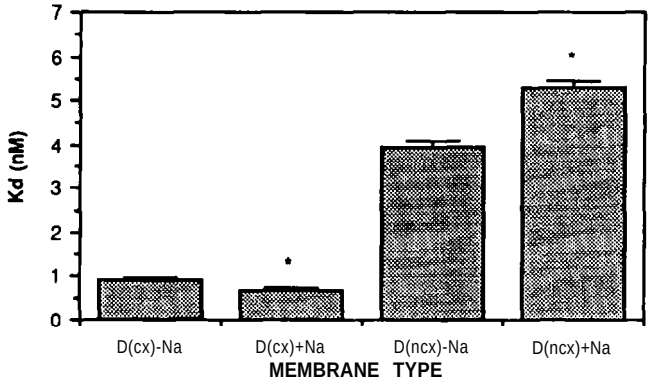
To determine if the morphine-induced upregulation of the δ_{cx} binding site is linked to the development of tolerance and dependence, rats were administered (i.c.v.) 20 nmol of the irreversible u-antagonist ß-FNA 60 min prior to pelleting. As reported in Figure 4, although ß-FNA did not effect the Bmax of the δ_{cx} binding site, it did prevent the upregulation.

DISCUSSION

Although current dogma holds that chronic morphine does not alter opioid receptors (Robson *et al.* 1983), the data reviewed in this paper demonstrate that both chronic morphine and chronic naltrexone upregulate opioid receptors. Since chronic morphine increases the Bmax of the δ_{cx} site, and not the δ_{ncx} binding site, it would be difficult to detect the approximately 20% increase in the total number of δ binding sites. However, our use of site-directed irreversible ligands permitted us to detect the change occurring in one site using a ligand which labeled two binding sites.

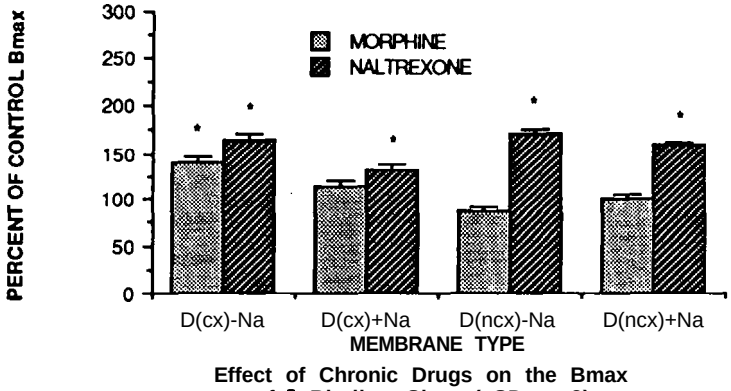
In the first series of experiments, the effects of the Na-preincubation on δ_{cx} and δ_{ncx} binding sites were determined. As shown in Figure 1, Na-preincubation decreased the Kd of the δ_{ncx} binding site, and increased the Kd of the δ_{cx} binding site. Na-preincubation had no significant effect on the Bmax values-data not shown . In subsequent

Figure 1



Effect of Na-prelncubation on Kd values (mean±SE (n=6)}..

Figure 2

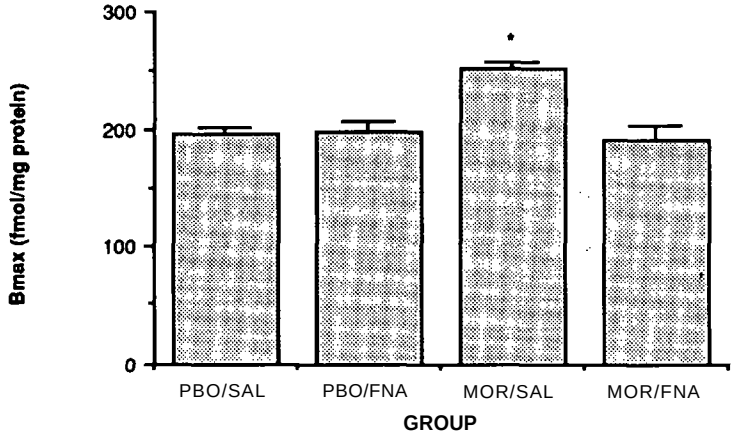


Effect of Chronic Drugs on the Bmax of δ Binding Sites (±SD, n=3)

experiments, the effect of chronic morphine and chronic naltrexone on the δ_{cx} and δ_{ncx} binding sites were determined using -Na- and +Na-membranes, and compared as a percentage of the control values.

As reported in Figure 2, chronic naltrexone increased the Bmax of the δ_{cx} and the δ_{ncx} binding sites, using both -Na and +Na-membranes. Chronic morphine increased only the Bmax of the δ_{cx} binding site

Figure 4



Effect of ß-FNA on morphine induced upregulation of δ Binding Sites (±SD, n=4)

chronic naltrexone did not. Similarly, chronic naltrexone increased the Bmax of the δ_{ncx} binding site whereas chronic morphine did not. Moreover, although the mechanism(s) by which the preincubation in

Table 2

Effects of Chronic Morphine and Chronic Naltrexone on Opioid Receptor Subtypes

	Kd				Bmax			
	δ_{cx}	δ_{ncx}	μ	κ	δ_{cx}	δ_{ncx}	μ	κ
Chronic Morphine	⇑	⇑	⇑	⇔	⇑	⇔	⇑	⇔
Chronic Naltrexone	⇔	⇑	⇔	⇓	⇑	⇑	⇑	⇑

Summary of the effect of chronic morphine and chronic naltrexone on the binding parameters of opioid receptor subtypes. (⇑ increase in value; ⇓ decrease in value; ⇔ no change in value).

If both drugs upregulated opioid receptors via the same mechanisms, then we should have observed identical changes in the binding parameters of the δ binding sites. Instead, the pattern of alterations in binding parameters differed (Table 2). For example, chronic morphine increased the Kd of the δ_{cx} binding site whereas 0.4 M NaCl alters

binding parameters is not known, the fact that the δ_{CX} sites upregulated by chronic morphine are labile to this procedure, and those upregulated by chronic naltrexone are not, supports the hypothesis that chronic morphine and chronic naltrexone upregulate opioid binding sites by different mechanism(s).

Although the studies described above established that chronic administration of morphine upregulates the opiate receptor complex, they do not directly address the hypothesis that the observed upregulation is a relevant in vitro marker of the development of tolerance and dependence. Although the acute administration of morphine has no effect on the δ_{CX} binding site (Danks *et al.* 1988), it remained a possibility that chronic morphine would upregulate the opiate receptor complex even if tolerance and dependence were not to develop. Such a finding would suggest that the observed upregulation is not related to the phenomena of tolerance and dependence.

To test this hypothesis, rats were administered ß-FNA, prior to and during morphine administration, a drug we observed to attenuate the development of tolerance and dependence. If the observed upregulation is linked to the development of tolerance and dependence, the predicted result would be an attenuation of the upregulation. The observation (Figure 4) that ß-FNA administration blocked the upregulation supports the hypothesis that morphine-induced upregulation of the opioid receptor complex is a relevant biochemical marker of tolerance and dependence.

Although it is tempting to discard these observations on the basis of now classic studies conducted in the adrenergic systems (Creese, and Sibley, 1981) a more fruitful approach is to hypothesize that the mechanism(s) responsible for the development of tolerance and dependence to morphine in the CNS are more complex than previously thought on the basis of investigations carried out in simpler model systems (Sharma *et al.* 1975; Chavkin, and Goldstein, 1984). Models of tolerance and dependence must explain three phenomena which are generally accepted to be inseparable consequences of chronic morphine: 1) agonist subsensitivity (tolerance), 2) dependence, and 3) antagonist supersensitivity (as the degree of tolerance increases, less naloxone is required to produce withdrawal (Way *et al.* 1969; Wei *et al.* 1973)).

Based in part on our results, we postulated an anti-opiate model of tolerance and dependence (Rothman *et al.* 1986a; Danks *el al.* 1988; Simonds, 1988), which hypothesizes morphine causes release of endogenous anti-opiate peptides, which are largely responsible for the development of tolerance and dependence. Recent data that the chronic administration of morphine downregulates the MIF receptor (an anti-opiate receptor) (Zadina *et al.* 1989) suggests that this anti-opiate might play a major role in morphine tolerance and dependence.

Because of the space limitations of this paper, we refer the reader to (Rothman *et al.* 1989) for more complete description of this model.

Although speculative, this model provides for many testable hypotheses. It is hoped that morphine-induced upregulation of the opiate receptor complex will prove to be a useful in vitro marker with which to examine the anti-opiate hypothesis of morphine tolerance and dependence.

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Reference list available upon request from R.B.R.

AUTHORS

Richard B. Rothman M.D., Ph.D., Unit on Receptor Studies, LCS, NIMH, Bethesda, MD. 20892.

Joseph B. Long, Ph.D., Neuropharmacology Branch, Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Victor Bykov, Unit on Receptor Studies, LCS, NIMH, Bethesda, MD, 20892.

Kenner C. Rice, Ph.D., Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

John W. Holaday, Ph.D., Scientific Director, MEDICIS CORP.,1747 Pennsylvania Ave., N.W., Washington, DC 20006.

Mu Antagonist and Kappa Agonist Properties of b-Funaltrexamine (b-FNA): Long Lasting Spinal Antinociception

Qi Jiang, Julius S. Heyman and Frank Porreca

The multiplicity of opioid receptors has led to the development of novel and irreversible probes to facilitate the study of the effects mediated by individual receptor subtypes. One such compound ß-funaltrexamine (ß-FNA)(Portoghese et al., 1980). Using the electrically stimulated guinea pig ileum longitudinal muscle preparation and-the mouse vas deferens as bioassays *in vitro,* ß-FNA has been characterized as a reversible κ agonist and an irreversible μ antagonist (Takemori et al., 1981; Ward et al., 1982b,c). Antinociceptive studies in the mouse *in vivo* (acetic acid-induced writhing test and tail flick test) and studies of gastrointestinal propulsion as pharmacologic endpoints are consistent with the conclusion that ß-FNA acts as a reversible κ agonist and as an irreversible μ antagonist if the data are interpreted in light of findings *in vitro* (Ward and Takemori 1983a,b).

A growing body of evidence suggests, however, that some opioids classified as κ preferring may act as μ antagonists in systems where they exert no agonist actions when given alone. For example, κ agonists such as ethylketocyclazocine and MR2034 have been found to be potent antagonists of [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAMGO) and [D-Ala^2, D-Leu^5]enkephalin (DADLE) in the field-stimulated rat vas deferens (Gillan et al., 1981). Additionally, the κ agonists U50,488H, tifluadom. ethylketocyclazocine and MR2034 were found to consistently antagonize the increase in striatal dopamine metabolism and respiratory depression produced by morphine (Wood 1984). In recent studies using μ opioid inhibition of rat urinary bladder motility, Sheldon et al. (1987,1989) found that κ agonists such as U50,488H. ethylketocyclazocine and tifluadom, as well as dynorphin A-(1-17) antagonized the effects of some μ agonists. such as morphine. Additionally, the antagonism of morphine by dynorphin A-(1-17), but not the direct agonist effect of morphine, was blocked by the κ antagonist, nor-binaltorphimine (nor-BNI)(Takemori et al., 1988). suggesting that dynorphin A-(1-17) modulated morphine effects through a κ receptor (Sheldon et al., 1989).

In the pharmacological characterization of ß-FNA *in vivo* Ward and colleagues allude to the fact that the agonist actions of ß-FNA in the mouse writhing test are present up to 48 hr after subcutaneous (s.c.) administration of the compound (Ward et al., 1982a; Ward and Takemori 1983a,b); this time course parallels closely, that of ß-FNA to antagonize the antinociceptive effects of morphine, a μ agonist. in the tail flick test (Ward et al., 1982a). Additionally. it is often difficult to detect actions of κ agonists following direct administration into the brain or spinal cord, particularly in tests utilizing heat as the nociceptive stimulus (Porreca et al., 1984). Thus, it seemed possible that the μ antagonist properties of ß-FNA might be the result of its long-lasting agonist actions at the κ receptor, rather than by direct interaction at the μ site. The present study was designed to test this possibility using the mouse acetic acid-induced writhing test, a reliable indicator of central κ agonist actions (Porreca et al., 1987).

METHODS

Male, ICR mice (20-30 g. Harlan) were used for all experiments. Animals were kept in groups of 5 in temperature controlled room with a 12 hr light-dark cycle (lights on 7:00 A.M. to 7:00 P.M.). Food and water were available *ad libitum* until the time of the experiment. ***I.th.*** injections were made according to the modified method of Hylden and Wilcox (1980). Injections into the subarachnoid space between L5 and L6 were made to unanesthetized mice using a Hamilton microliter syringe fitted with a 30-gauge needle. All ***i.th.*** injections were made in a volume of 5 µl.

Acetic acid writhing test

The method used was as previously described (Porreca et al., 1987). Each mouse received ***i.th.*** administration of agonist, antagonist, or saline at various times prior to the intraperitoneal administration (***i.p.***) of acetic acid (0.6%. 10 ml/kg), and was then placed into an individual observation chamber. After 5 min, the mouse was observed for a further 5 min, and the number of writhes displayed by each mouse was counted. Percent antinociception was determined according to the following formula:
% antinociception = 100 x (mean number of writhes in control group - number of writhes for each test mouse)/(mean number of writhes in control group), where the control group was defined as all animals treated with ***i.th.*** saline.

Tail flick test

The thermal nociceptive stimulus was 55°C warm water with the latency to tail flick or withdrawal taken as the endpoint. After the determination of control latencies. the mice received graded doses of id. agonist, or antagonist at various times. ß-FNA was given ***i.th.*** as a single injection and testing took place 10 min and 1, 2, 4, 24, 48 and 72 hr later. In some antagonist studies, ß-FNA was given as a single pretreatment dose at various times up to 48 hr prior to testing. Nor-BNI was always given 10 min prior to the agonist, or in the case of pretreatment with ß-FNA. at 10 min prior to testing. A cutoff time of 15 sec was employed; if the mouse failed to demonstrate a tail-flick, the tail was removed from the water and that animal was assigned a maximum score. Mice not responding within 5 sec in the initial control trial were eliminated from the experiment. Antinociception at each time point was calculated according to the following formula % antinociception = 100 x (test latency - control latency)/(15 - control latency).

RESULTS

Both ß-FNA and U50,488H produced dose-related antinociception when tested 10 min after *i.th.* administration in the writhing test (Figure 1a). While ß-FNA produced a long-lasting antinociception in this test (Figure 1b,c,d), U50,488H failed to produce significant antinociception after 1, 2. and 4 hr (Fig. 1b,c,d). The antinociceptive effect of ß-FNA was dose- and time-related up to 48 hr after a single ***i.th.*** administration. Nor-BNI (0.37 nmol, ***i.th.***) produced no agonist actions in either analgesic test, but administration 10 min prior to testing in mice pretreated with ß-FNA (at -10 min or -4 hr prior to testing) resulted in a rightward displacement of the ***i.th.*** ß-FNA dose-response curves in the writhing test; the ß-FNA lines were shifted 62 and 34 fold when the agonist was given 10 min or 4 hr prior to testing (Table I).

I.th. ß-FNA failed to produce agonist effects in the tail-flick test, while morphine was active in both analgesic tests. For this reason, the antagonist profile of ß-FNA could only be evaluated in the tail-flick test. In addition to its long-lasting agonist effects in the writhing test, ***i.th.*** pretreatment with ß-FNA (18 nmol at -12 hr) also produced a long-lasting antagonism of the analgesic effects of ***i.th.*** morphine in the tail flick test the ß-FNA pretreatment resulted in a 3-fold rightward displacement of the morphine dose-response line. Pretreatment with nor-BNI (-10 min. 0.37

nmol) did not antagonize the antinociceptive effects of morphine in the tail-flick test and additionally, did not block the antagonism of morphine by ß-FNA in this test. Further, *i.th.* nor-BNI (0.37 nmol at -10 min) did not antagonize the antinociceptive effects of *i.th.* morphine in the writhing test. When tested as an antagonist against *i.th.* morphine antinociception. the time course of *id.* ß-FNA antagonist actions in the tail-flick test showed an inverse relationship to that for *i.th.* ß-FNA agonist effects in the writhing test.

TABLE 1

Effects of *i.th.* ß-FNA in the mouse acetic acid writhing test. ß-FNA was given 10 min or 4 hr prior to testing in the absence and presence of *i.th.* nor-BNI (0.37 nmol); the K antagonist was *always* given 10 min prior to testing. Ten mice were used in each test group.

Dose ß-FNA (nmol)	control (± S.E.)	nor-BNI (± S.E.)	control (± S.E.)	nor-BNI (± S.E.)
0.0019	25.8 ± 6.8	—	—	—
0.019	42.5 ± 6.8	9.8 ± 7.3	21.2 ± 4.6	—
0.19	77.2 ± 12.2	39.5 ± 8.1	51.6 ± 9.4	20.0 ± 12.7
1.88	94.3 ± 3.9	54.6 ± 9.7	71.7 ± 10.7	50.0 ± 14.9
18.8	—	94.4 ± 5.6	—	63.2 ± 11.8

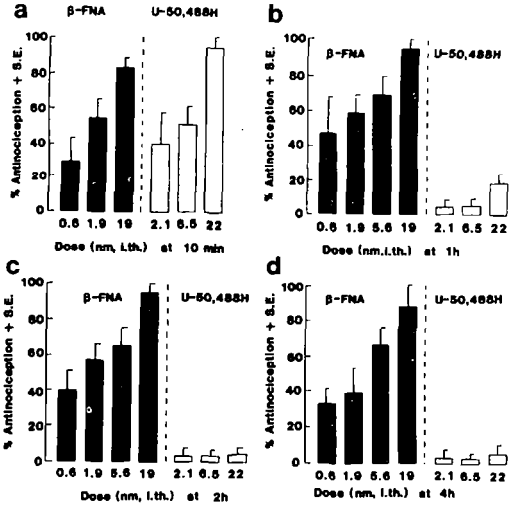

FIGURE 1

Antinociceptive effects of *i.th.* ß-FNA or U50,488H in the mouse acetic acid writhing test when given 10 min (a) or 1, 2 or 4 hr (b, c, d, respectively) before testing.

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DISCUSSION

Following the characterization *in vitro* of ß-FNA as a reversible κ agonist and an irreversible μ antagonist (Ward et al., 1982b,c). many studies have used this compound as a tool with which to characterize the receptor involvement of opioids in the production of effects *in vivo* such as antinociception (e.g., Hayes et al., 1986; Zimmerman et al., 1987). respiratory function (Ward and Takemori 1983c; Hayes et al., 1985a) and other actions such gastrointestinal propulsion (Ward and Takemori 1983b; Hayes et al., 1985a; Heyman et al., 1987) and development of physical dependence (Gmerek and Woods 1985; Aceto et al., 1986). Additionally, this compound has also been extremely useful in studies *in vitro,* in classifying actions of selective agonists in bioassays such as the mouse vas deferens and guinea-pig ileum (e.g., Ward et al., 1982b,c; Gintzler and Hyde 1984; Smith 1986; Hayes et al., 1985b). From these diverse studies, it is clear that ß-FNA has played a significant role in the development of current understanding of opioid actions *in vitro* and *in vivo.*

Analysis of the mechanism of ß-FNA actions, however, has been somewhat controversial. Radioligand binding studies in mouse brain, have suggested that ß-MA may bind in a reversible (preferring κ sites over μ or **δ)** and an irreversible (preferring μ sites over**δ** sites)(Ward et al., 1985) fashion. The irreversible nature of ß-FNA binding at the μ site was confirmed using rat brain (Tam and Liu-Chen 1986; Liu-Chen and Phillips 1987). and has been suggested to include both the μ_1, and the μ_2 binding sites (Recht and Pasternak 1987). In the course of studies designed to employ ß-FNA as a long-lasting μ antagonist at the spinal level, we observed that this compound produced long-lasting antinociception in the writhing test after *i.th.* administration to mice. Additionally, the time-course of the agonist activity of *i.th.* ß-FNA in the mouse writhing test paralleled closely its ability to antagonize morphine in the tail flick test. Coupled with other observations that κ agonists could, under some circumstances, act as μ antagonists (e.g., Wood 1984; Holaday et al., 1985; Tortella and Holaday 1987; Sheldon et al., 1987) and with the fact that the analgesic effects of centrally-given κ agonists can be difficult to detect in many tests (especially those emphasizing heat)(Porreca et al., 1987). it seemed possible that the long-lasting p-antagonist properties of this compound were related to its κ agonist actions. This concept was particularly intriguing as the receptor binding characteristics of ß-FNA had been determined using brain, rather than spinal cord, tissues. The present study has thus systematically examined and extended the pharmacology of this important opioid tool *in vivo using* two analgesic tests and administration of selective κ agonists and antagonists at spinal sites in the mouse.

We have focused on the actions of ß-FNA as an agonist or antagonist at the spinal level. Unlike administration of U50,488H, a highly selective κ opioid agonist (Von Voigtlander et al., 1983), ß-FNA produced long lasting antinociceptive effects after *i.th.* administration in the writhing test. The antinociceptive actions of *i.th.* ß-FNA were shown to be κ mediated in that they were blocked by the κ antagonist, nor-BNI (Takemori et al., 1988) whether ß-FNA was given either either 10 min or 4 hr prior to testing. ß-FNA was not effective in-the tail-flick test following *i.th.* administration, the nature of its antagonist actions could also be investigated. Pretreatment with ß-FNA but not nor-BNI, produced a rightward shift of the *i.th.* morphine dose-response line. *I.th.* nor-BNI did not, however, prevent the antagonism of morphine produced by *I.th.* ß-FNA indicating that the antagonist actions of this compound were not related to its κ agonist properties, and thus were probably mediated through actions at the μ receptor. On the basis of the present experiment, we conclude that *i.th.* ß-FNA produces its long-lasting agonist actions through κ receptors, and that its antagonist properties are most likely the result of activity at μ receptors. The nature of its long lasting interaction at these receptors appears to be similar, however, in that the agonist and antagonist time-course are similar.

In conclusion, this study has demonstrated long-lasting agonist actions of *i.th.* ß-FNA in an analgesic test emphasizing chemical stimulation. Additionally, at the spinal level, the antagonist profile of this compound appears to be mediated through different receptors than its agonist actions. These experiments suggest a complex mechanism of action for this compound and indicate that the possibility that ß-FNA antagonizes μ agonists by its own agonist action at κ receptors cannot be supported.

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ACKNOWLEDGEMENTS

This work was supported by DA 04285.

AUTHORS

Qi Jiang, M.D.
Department of Pharmacology
University of Arizona Health Sciences Center
Tucson, AZ 85724, U.S.A.

Julius S. Heyman, Ph.D.
Department of Pharmacology
University of Arizona Health Sciences Center
Tucson, AZ 85724, U.S.A.

Frank Porreca, Ph.D.
Department of Pharmacology
University of Arizona Health Sciences Center
Tucson, AZ 85724, U.S.A.

Urine Screening: What Does it Mean?

D. E. McMillan

Surveys conducted by the National Institute on Drug Abuse suggest that men and women of working age frequently use psychoactive drugs (NIDA, 1986) and it seems likely that a considerable portion of this drug usage occurs on the job. One recent survey found that one in ten of all job applicants and employees tested positive for drugs (U.S. Department of Labor, 1989). Estimates of the dollar cost of workplace drug abuse suggest that the cost of drug abuse in the workplace may be as much as $700 billion (Walsh and Gust, 1986). Although data on both prevalence and costs are imprecise,
there is general agreement that drug usage at the workplace is a serious problem, not only in terms of dollar costs, but also in work-related accidents, job dismissal, illness and mortality.

In response to the problem, the Reagan administration initiated a broad based program with an ultimate goal of a "drug-free workplace" with the federal workforce serving as a model for American business. The President's Executive Order directed federal agency heads to develop policies on drug abuse, including employee assistance programs (EAPs), training programs and a drug testing program. Since that time a large number of private companies in the United States have initiated programs designed to eliminate drug use in the workplace, the centerpiece of which is frequently a drug testing program. These programs have been widely publicized and exceedingly controversial for legal, moral and scientific reasons. To set the stage for later papers, I would like to review briefly some of these issues about which you will be hearing in greater detail later in the symposium.

A. ANALYTICAL AND PHARMACOLOGICAL CONSIDERATIONS

The consequences of screening the urine of a worker or job applicant for illegal drugs include refusal to hire, dismissal from the workforce and other forms of discipline, loss of the worker's reputation and other punishments, as well as more positive outcomes, such as referral to treatment. Because the consequences of a positive screen are so important, the chemical analysis of the urine must be absolutely accurate.

An adequate drug test usually consists of two parts: an initial screening test and a confirmation test when the initial screen is positive. A number of initial screens are available, including the EMIT, ADX and TDX. These screens have the advantages that they are highly automated, reasonably specific and relatively inexpensive. Thus, they permit the rapid and inexpensive screening of large numbers of urine specimens. Unfortunately, reasonably specific is not adequate when the consequences of a positive drug test are so important. Therefore, these initial screening tests are used only to enable the testing laboratory to eliminate drug negative specimens from further consideration, while positive specimens must be validated by a confirmation test.

The "gold standard" of confirmation tests is gas chromatography-mass spectrometry (GC/MS), a procedure with a high degree of analytical accuracy. Although the GC/MS test is more difficult and expensive than initial screens, confirmation of a positive screen with this test is an extremely reliable index of drug exposure. It is most unfortunate that with the widespread proliferation of drug testing programs many companies do not perform any confirmation testing (U.S. General Accounting Office, 1988; Thomas-Holladay et al., 1989). Failure on the part of a company to perform a confirmation test probably results from ignorance on the part of management as to the importance of a confirmation test and a desire to do drug testing at the lowest possible cost. Obviously, this can be a false economy, since a positive drug test consisting only of an initial screen is subject to legal challenge.

Although an initial screen, followed by a GC/MS confirmation, under conditions of a carefully documented chain of custody appears to be quite straightforward, in practice this is not necessarily so. Under the Mandatory Guidelines for Federal Workplace Drug Testing Programs cutoff points have been established for both the initial screen and the GC/MS confirmation (Federal Register, 1988). The need for cutoff points arises because our increasing analytical capacity has resulted in our ability to measure ever smaller amounts of drug or drug metabolites in urine. Theoretically, the ultimate level of analytical precision might be reached when a single molecule of drug or drug metabolite can be detected in body fluids. Obviously, at this extreme the accidental inhalation of a few molecules of marijuana smoke would be detected. Although we have not yet reached this level of precision and sensitivity, our ability to measure drug exposure has probably outstripped our ability to interpret the results.

Cutoff points have been recommended in the Mandatory Guidelines (Federal Register, 1988) which are designed to eliminate problems such as passive inhalation of marijuana smoke. Unfortunately, private companies are not required to follow these federal guidelines and many do not. For example, in a six state survey of union workplaces in the midsouth, only 24% reported that their company used the guidelines (Thomas-Holladay et al., 1989). In our experience in the Occupational Substance Abuse Prevention Program at the University of Arkansas for Medical Sciences we have found that companies often have rational reasons for adopting cutoff points other than those recommended by the federal guidelines. For example, a company may institute a policy that no contract worker can go on a worksite without a drug-free" urine. When a worker's urine is positive in the initial screen and negative on confirmation, the company may have

to pay the contractor's workforce during the period between the screen and the availability of the confirmation test Thus, the company might have economic reasons for wanting to have most positive screens confirmed in the GC/MS test. Toward this end the company might set a high cutoff point in the initial screen and a low cutoff point in the GC/MS confirmation.

Some companies can have difficulty in understanding the results reported to them. According to the Mandatory Guidelines, results can be reported as negative or positive, but the Medical Officer may request a quantitation of the test results (Federal Register, 1988). In our program we nave found that most Medical Officers do want a quantitative report; however, with small companies, the Medical Officer is frequently a part-time general practitioner, or perhaps an occupational nurse, with little or no experience in interpreting drug testing data. For example, the Mandatory Guidelines recommend 100 ng/ml as a cutoff point for the initial screen and 15 ng/ml for the GC/MS confirmation for marijuana. The initial screen shows various degrees of cross reactivity to a variety of Δ^9-tetrahydrocannabinol (THC) metabolites. The method has a sensitivity down to 10 ng/ml for combined cannabinoids to which cross reactivity occurs. The GC/MS is for a specific Δ^9-THC metabolite, Δ^9-THC-9-carboxylic acid, which can be measured reliably at levels of 2 ng/ml. Thus, both initial and confirmatory assays are sensitive well below the recommended cutoff points. How a company interprets and uses this quantitative information is frequently beyond the control of the testing laboratory.

Perhaps the greatest source of confusion in drug testing programs is the meaning of a positive report. The detection of a drug in the urine of a worker does not imply that the worker was impaired at the time of testing, or even that the worker ever was impaired. A positive result is indicative only of exposure, not pharmacological effect This should not be surprising since the recommended cutoff points for initial screens for marijuana, cocaine and opiates are based on metabolites rather than the parent drug and the GC/MS analysis for marijuana and cocaine are also based on metabolites. Many of these metabolites are not psychoactive. Furthermore, these metabolites are frequently detectable long after the psychoactive effects of the drug have terminated.

Closely related to the issue of screening for detection of exposure to a drug versus impairment of performance is the question of whether or not a confirmed positive drug screen means that the worker has a drug "problem". Some companies feel that one confirmed positive drug screen is adequate evidence for action, while others may require repeated positive tests, or evidence of impaired performance.

A final issue that should be mentioned is that drug testing programs are biased toward the detection of drugs or metabolites that remain in the body for long time periods. For example, cocaine metabolites are detectable in the body for only a few days, while metabolites of marijuana can be detected for a month or more in the urine of chronic users. Thus, a drug testing program is more likely to detect the weekend marijuana smoker than the weekend cocaine user. Which of these workers represents the greatest problems in the workplace is not clear.

B. COST EFFECTIVENESS

The basic assumption underlying the screening of the workforce for exposure to illegal drugs is that the screening program is an effective deterrant to drug use. According to recent surveys, drug usage (during the past month) declined in the military by over 80% during the 8-year period after drug screening was initiated (NIDA, 1988). Whether or not similar decreases in exposure can be demonstrated in private industry seems less clear and the degree to which the drop in drug usage by the military has improved military performance is also unclear.

At this time there is a strong movement toward development of drug screening programs in American companies. These programs are not without financial costs. Depending on the particular test and the number of workers to be tested, drug screens cost in the range of $15 - $30 per specimen and GC/MS confirmation generally costs from $35 - $100. Thus, the screening of 100 million workers might cost about $2 billion based on a 10% rate of positive initial screens and average price levels. This figure represents a very small fraction of some estimates of losses due to substance abuse; however, estimates of losses due to substance abuse are based on soft data and there have been very few studies that have attempted to measure the cost effectiveness of drug testing programs. Do drug testing programs really reduce employee drug use and does the reduction of employee drug use lead to decreases in accidents, absenteeism, utilization of treatment resources and increases in productivity? It is a fundamental assumption in drug testing programs that a drug-free workplace will increase productivity and decrease costs, but there are few data available that compare the savings produced by a testing program with the costs of the program.

C. LEGAL AND MORAL CONSIDERATIONS

Some individuals have held that mandatory drug testing is a violation of an individual's civil rights. In general, courts have upheld the employer's right to screen job applicants for drug use and to screen workers involved in accidents or other work incidents for evidence of drug usage. The random screening of selected employees or all employees is less well established. Certainly everyone agrees that the worker has certain rights and that the worker's dignity should be protected.

Although the Mandatory Guidelines (Federal Register, 1988) specify that an employee with a confirmed positive drug test must be referred to an employee assistance program (EAP), in private industry many companies make little attempt to meet the federal guidelines (Thomas-Holladay et al., 1989). Many smaller companies do not even have an EAP to which to refer workers and frequently punitive actions result from positive drug tests up to and including job dismissal. In our program we nave been reluctant to work with companies that do not refer current employees with positive drug tests to the EAP; however, the policing of private industry is not a burden appropriate for testing laboratories to assume and for those testing laboratories that feel squeamish about the use to which their testing results might be put, it is likely that other testing laboratories with less concerns about what happens to the worker will be only too ready to perform the testing.

These issues are very complex. Certainly the public has a right to expect that those workers with jobs critical to public safety are not using performance-impairing drugs that compromise public safety. Similarly, the company hiring a worker would like to know that that worker does not have a serious drug problem and the company owes it to its stockholders to reduce any factor that increases costs and lessens productivity, including illegal drug use. However, the company also has a responsibility to the impaired employee to refer him to a treatment facility. Finally, the worker also has a right to his or her dignity and to freedom from unnecessary harassment and discipline by management.

For better or for worse, the industrial base of this nation, with the encouragement of the federal government has embarked on a massive experiment in drug testing. They are doing so with little education of either management or the workforce as to the meaning of the data being provided, inadequate determination of the cost effectiveness of the approach and with important legal and moral questions as yet unanswered. It is important that scientists, physicians, lawyers, ethitists, economists and others concerned with these complex problems work together to find answers to some of these questions.

The next series of papers will assess the state of knowledge in some of these areas. Dr. Royer Cook will provide further details about the prevalence of drug abuse in the workplace. Dr. Dennis Crouch will review research on drug testing program effects on absenteeism, accidents and costs. Captain Leo Cangianelli will report on the experience of the military with their drug testing program. Professor Eleanor Schroeder will update the current legal status of drug testing. Finally, concluding remarks and a summary will be made by Dr. John Grabowski.

REFERENCES AVAILABLE ON REQUEST

AUTHOR
D.E. McMillan
Department of Pharmacology and Toxicology
University of Arkansas for Medical Sciences
4301 West Markham St. - Slot 611
Little Rock, Arkansas 72205

The Effects of a Drug Testing Program in the Navy

Leo A Cangianelli

HISTORICAL OVERVIEW

Two significant incidents early in this decade had a great impact on the U.S. Navy's resolve in fighting its "War on Drugs":

In November 1980, the Department of Defense (DOD) conducted the first of four surveys to investigate the prevalence of drugs in the military and the consequences on work performance and health.

The second incident took place in the spring of 1981, when results of autopsies on fourteen sailors killed in a crash of an EA-6B Prowler on the deck of the USS NIMITZ revealed that six of the flight deck crew had traces of marijuana in their blood system.

Each incident effected a measured response on the part of the Navy: the first started the development of a well thought out long range program; the Nimitz crash greatly accelerated this program.

The First DOD Survey

The results of the survey, titled "The Burt Study,"[1] revealed a shockingly high incidence of drug use [2] in all the services with marijuana use of epidemic proportions. The highest prevalence was in the Navy and Marine Corps: 47 percent of E-l to E-5 Navy personnel (essentially those junior members under 25 years of age) had used marijuana in the thirty days prior to completing the survey questionnaire.

The survey also revealed that drug use was not an off-duty problem -- 26 percent of the junior enlisted reported being under the influence of drugs while at work, and nearly one-half of these respondents reported using drugs on 40 or more occasions in the previous year. To Navy leadership, this latter result was particularly devastating because of the obvious impact on combat readiness and national security.

To verify the survey statistics, the Navy conducted random urinalysis testing on a similar group of sailors in Norfolk, Virginia, and San Diego, California, and confirmed the findings of the DOD report.

"Zero Tolerance" Edict

In response to the survey results, Chief of Naval Operations, Admiral Thomas B. Hayward, tasked his drug prevention staff with developing a strong program aimed at "Zero Tolerance" of drug use and stressed that the Navy's renewed emphasis on pride, professionalism and readiness, was incompatible with drug use. He promulgated the "get tough" policy in July 1981 in a message, NAVOP 097-81, in which he admonished commanding officers and supervisors that the illegal use of drugs, regardless of the degree of acceptance in the civilian sector, would not be condoned.

This policy contrasted with the Navy's previous policy of depending heavily upon treatment and education programs to deal with the problem.

The USS NIMITZ Incident

Media treatment surrounding autopsy results of the six flight deck crew members, the second incident impacting the "War on Drugs", caused public outrage. Congress demanded that the Navy take a tougher stance on drug use.

Ironically, at the time of the NIMITZ crash and the adverse publicity, the Navy was involved in long-range planning to combat drug use. This plan was not altered but was accelerated: the planned three-year implementation of the program was speeded up and was in place in one year.

In December 1981, Admiral Hayward launched his three-pronged offense against drug use and announced the goal of "Zero Tolerance": (1) any unauthorized use of illegal drugs, including marijuana, would be considered a serious breach of discipline; (2) violators would receive stiff penalties including courts martial and administrative "bad paper" discharges; and (3) urinalysis testing aimed at detecting, identifying, and deterring drug abusers, would be implemented on a wide scale.

The detection and deterrence program and Admiral Hayward's hard-nosed, get tough policy to stamp out drug use was announced in a special videotaped message sent fleetwide. The famous expression, "Not on my watch! Not on my ship! Not in my Navy!" was first decreed in his video message and became the "battle cry" for the program.

NAVY POLICY

The new Navy policy was promulgated in two messages issued on December 23, and 29, 1981 (NAVOPS 172-81 and 178-81). The program commenced one month later on February 1, 1981, and remains the same today with only minor changes. Essentially, the policy contained in the two directives states:

> "The illegal possession, use, and distribution of drugs and drug paraphernalia are not tolerated in the U.S. Navy at any time: ashore, afloat, on or off duty, on or off base ...It is Navy policy to make full use of urinalysis testing to assist in controlling drug abuse...." Policy requires that senior personnel be separated after a single incident of drug use. Junior personnel may be allowed a second chance if they demonstrate exceptional potential for continued service.

URINALYSIS TESTING

Implementation of the policy places emphasis on urinalysis testing of new recruits at the point of entry within 48 hours of their arrival at "boot camp" and again immediately upon arrival at the first training school. Processing for separation is mandated for individuals who produce an initial "positive" for any drug other than marijuana. Those testing positive for marijuana may be retained and randomly tested once each month for six months. A second positive for any drug results in processing of an individual for separation.

A confirmed positive urinalysis test at entry is considered a first drug use incident. A second drug use incident is cause for mandatory processing for separation.

This intensive testing program early in the member's career is designed to demonstrate a strong commitment by the Navy to identify and eradicate drug abuse.

Once a sailor reports to his or her first duty station, the member is subject to the command urinalysis screening program wherein 20 percent of each unit is tested every month. Most of these are random tests but testing can be conducted incident to a search and seizure, for probable cause, during medical examinations or to determine fitness for duty.

In addition to urinalysis testing, commanders use every means available to identify individuals involved in any way with illegal drugs. This includes special investigative agencies, on and off base, to identify traffickers supplying illegal drugs to Navy personnel; drug detection dog searches to locate and identify drug "stashes"; health and welfare inspections; and randomly searching vehicles for contraband. Traffickers are court-martialed and placed in pretrial confinement.

Leaders set the example. Any use or possession of illegal drugs by leaders is considered reprehensible and totally inconsistent with the exemplary role they play in the Navy. Consequently, officers and senior petty officers who use or possess drugs are processed for separation at the first drug incident. Junior enlisted personnel may be afforded a second chance provided they totally reject the drug use and show potential for continued drug-free naval service. Following a first drug use incident, junior enlisted personnel are disciplined, educated or counselled, and generally returned to duty following successful rehabilitation.

Collection and Laboratory Testing

Random samplings and unit "sweeps" are two ways of conducting urinalysis inspections in the Navy and serve as strong deterrents to drug use. In the random sampling program, individuals selected for testing are chosen at random through combinations of social security numbers, computer number selections, etc. A unit sweep is the testing of an entire unit or a subunit -- an identifiable segment or class of a unit -- e.g., a division, barracks, all E-4 and below, all officers, all personnel who have reported for duty in the last month, etc. The urinalysis program is designed so that a member's chance of selection, and therefore detection, remains constant throughout the individual's enlistment.

A sample of at least 60 milliliters of urine is provided under the direct observation of a designated individual. The observer signs a ledger certifying that the specimen bottle contains the sample provided by the member and that it has not been tampered with in any way. The submitting member signs the ledger and initials the label on the specimen bottle. The coordinator initials the label and records the information on a Urine Sample Custody Document (OPNAV 5350/2). The coordinator prepares the sample for shipment to the cognizant drug screen laboratory.

The Navy Drug Testing Laboratory system tests over two million specimens annually. Each sample is tested for seven drug classes at five locations: Great Lakes, Il., Jacksonville, Fl., Norfolk, Va., Oakland, Ca., and San Diego, Ca. The drug classes and cutoff levels are as follows:

DRUG	SCREENING LEVEL	CONFIRMATION LEVEL
THC (MARIJUANA)	100 NG/ML	15 NG/ML
COCAINE	300 NG/ML	150 NG/ML
OPIATES:		
MORPHINE	300 NGIML	4000 NG/ML
CODEINE	300 NG/ML	2000 NG/ML
HEROIN (6 MAM)	300 NG/ML	10 NG/ML
AMPHETAMINES	1000 NG/ML	500 NG/ML
PCP	25 NG/ML	25 NG/ML
LSD	.5 NG/ML	.4 NG/ML

When the sample reaches the drug testing laboratory, the specimen packages are inspected, the specimen bottle is numbered, and a sample is poured into the testing vials. Samples are screened using the radioimmunoassay (RIA) method which tests for a class of drugs; negative samples are eliminated from further testing.

From samples that test positive, two additional aliquots are poured and forwarded for simultaneous RIA screening and confirmation testing by the gas chromatography/mass spectrometry (GC/MS) equipment. The testing data for each positive sample are summarized, evaluated, reviewed within the lab, and only when all three tests (two RIA and one GC/MS) prove the sample to be positive above the cutoff levels are the results reported back to the submitting command.

To assure consistency across the entire system, the labs function under a centrally-managed, standardized operating procedure controlled by the Naval Medical Command. Quarterly inspections are conducted to enforce this policy and each lab conducts an intensive internal quality control program which requires that at least 20 percent of the samples tested in the lab are for quality assurance.

In addition, a centrally-monitored external quality control program is administered by the Armed Forces Institute of Pathology (AFIP) and closely monitored by both line and medical leadership.

External quality control is conducted in two separate and distinct procedures. The "blind" sample program imposes 48 samples per week on each laboratory--36 of which are negative and 12 of which are positives. These samples are provided by the AFIP directly to certain designated field commands who place their own label with an AFIP SSN on the bottle and include these samples with their own collected specimens. The entire package is submitted to the designated laboratory for testing. Results are monitored by AFIP and Navy line leadership. In the "open" sample program 24 spiked samples are provided to each lab for testing. In this case the lab must correctly identify the drugs and levels for each sample. Since 1982, when this program was implemented, none of the labs have reported a "false positive" of 6,000 negative blind samples submitted annually.

Each year, Navy line leadership conducts a full inspection of each laboratory with assistance from at least two leading civilian forensic toxicologists. This inspection has opened Navy labs for external technical scrutiny by the scientific world and has paved the way for Navy to become the model program for drug forensic testing.

EDUCATION AND TREATMENT PROGRAMS

In addition to a wide variety of command education and prevention programs, treatment and counselling programs are available worldwide and are designed to deal with all types of drug use problems from experimental use to full drug dependency. Four free-standing and twenty hospital-based residential alcoholism rehabilitation facilities are among the best in the world annually treating over 6,000 Navy and Marine Corps personnel. The intensive, six-week multi-disciplinary therapeutic program includes counseling, education, and participation in self-help groups. Over 85 Counseling and Assistance Centers provide screening, referral, outpatient counseling, and aftercare to Navy members whose problems do not require the inpatient treatment.

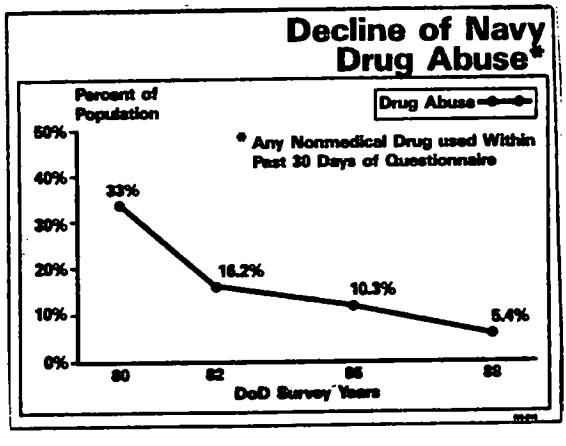
CONCLUSION

A program of this magnitude is not problem free. While there is close management and oversight of the laboratory process, urine sample collection procedures are the weakest link.

Over 2,000 Navy commands are responsible for collecting samples under chain of custody procedures which must be followed precisely. These procedures are explicitly outlined in Navy policy directives; a handbook provides guidance for urine collection teams.

Experience has shown that drug users will go to great lengths to defeat urinalysis testing including substituting clean urine, adulteration of the sample with foreign substances, and dilution with water. Direct observation and close supervision of the collection process, and attention to detail during the chain of custody process, have proven the best countermeasures.

The Navy's program is a resounding success. Follow-on DOD surveys in 1982, 1985, and 1989, have shown that drug prevalence has been reduced from 33 percent in 1980 to 5.4 percent today.



The "Zero Tolerance" policy remains the cornerstone of the program. Sixty four percent of respondents in follow-on surveys agreed that urinalysis testing has reduced drug use in the military and 81 percent believed that the program has not hurt morale. 3

The Navy's urinalysis program remains a national standard for a sound, valid, and legally-supportable testing plan. Analysis indicates that the multi-faceted approach of urine testing for identification and deterrence, appropriate application of discipline, and referral for treatment and counseling when relevant, are the essential features of a successful drug prevention and control program.

FOOTNOTES

[1]Subsequent DOD studies, titled "The Worldwide Survey of Alcohol and Nonmedical Drug Use Among Military Personnel," were conducted in 1982, 1985 and 1988. To compile the statistics for the 1988 DOD survey, about 19,000 questionnaires were mailed to selected installations worldwide requesting anonymous responses. To provide correlations and consider significant predictors of drug use, a model of any drug use during the previous 12 months was incorporated into the survey questionnaire. Conclusions are reached after extensive computer analysis and review by survey teams.

[2]"Drug use" is defined as use of marijuana, cocaine, PCP, LSD, or other hallucinogens, heroin or other opiates, inhalants, "designer drugs" or other non-medical drugs.

[3]1985 Worldwide Survey of Alcohol and Nonmedical Drug Use Among Military Personnel, Research Triangle Institute, June 1986, Page 154.

AUTHOR

Captain Leo A. Cangianelli, U. S. Navy, is the director of the Navy's Drug and Alcohol Abuse Prevention and Control Division, Washington, DC.

Legal Aspects of Urine Screening

Elinor P. Schroeder

Federal initiatives such as President Reagan's 1986 Executive Order 12,564, requiring the head of each Executive agency to establish a drug testing program, and Public Law 100-71, 101 Stat. 468 (1987), governing the scope of Federal Government drug testing, have been the driving force for employment drug testing in this country. That fact, plus the availability of constitutional challenges to government's actions, make it not surprising that most of the judicial analysis of drug testing programs has involved the public sector. The outcome of this litigation will, however, strongly influence developments in the private sector as well.

FEDERAL LAW

Fourth Amendment

On March 21, 1989, the United States Supreme Court upheld the constitutionality of two different federally mandated employment drug testing programs. While the Court did not give a blanket approval to drug testing under any and all circumstances, the sweeping language of these opinions, allowing testing of workers without individualized suspicion of drug use, will undoubtedly be used to justify many programs. In Skinner v. Railway Labor Executives' Association. 109 S. Ct. 1402 (1989), by a vote of seven to two, the Court upheld regulations promulgated by the Federal Railroad Administration requiring railroads to conduct blood and urine tests of employees involved in serious train accidents and authorizing railroads to conduct breath and urine tests after certain rule violations. In National Treasury Employees Union v. von Raab, 109 S. Ct. 1384 (1989) , by a vote of five to four, the Court held that the Customs Service did not violate the Fourth Amendment by requiring a urine test of applicants for positions directly involving drug interdiction or enforcement of drug laws and for positions requiring the carrying of a gun. The Court found the record inadequate to evaluate a testing requirement for a third category of positions, those involving the handling of "classified" material, and it remanded that portion of the case for further findings.

In both cases, the Court first cane to the fairly unremarkable conclusion that urine tests are "searches" within the meaning of the Fourth Amendment, because they infringe upon long-established, widely-recognized, and serious expectations of privacy. Traditional Fourth Amendment analysis requires that the government have probable cause and obtain a warrant before proceeding with a search. There are, however, some exceptions to the warrant requirement, when "special needs, beyond the need for law enforcement," make obtaining a warrant impracticable, New Jersey v. T.L.O., 469 U.S. 325, 351 (1985). The court determined that there was no need for the protection a warrant provides from arbitrary government action in either Skinner or von Raab because the tests were standardized and those in charge of the program had little discretion in selecting individuals to be tested. Moreover, in Skinner, the fleeting nature of the metabolic evidence required that tests be performed immediately, without the delay entailed in seeking a warrant. Noting the possibility that the results of a post-accident drug test might be turned over to law enforcement authorities, the Court left "for another day" the question whether routine use of test results in criminal proceedings would present a problem. 109 S. Ct. at 1415 n.5.

As to the Fourth Amendment's requirement of probable cause, although the Court has remarked that probable cause is not an "irreducible requirement" of a valid search, New Jersey v. T.L.O., supra at 340, until Skinner and von Raab. the search of an individual other than a prison inmate had never been upheld without some level of individualized suspicion. See New Jersey v. T.L.O., supra (search of student's purse by public school principal after the student was caught smoking in the school bathroom); O'Connor v. Ortega, 480 U.S. 709 (1987) (search of public employee's office, desk, and files as part of investigation of specific financial improprieties); Bell v. Wolfish. 441 U.S. 520 (1979) (suspicionless body cavity searches of prison inmates).

Actually, Skinner could have been shaped to fit this requirement of individualized suspicion. The FRA regulations required mandatory testing of all crew members on a train involved in a serious accident, even if some were not suspected of having played a part in the accident. The government presented evidence of significant problems of alcohol and drug abuse among railroad workers, including 34 fatalities, 66 injuries, and over $28 million in property damage during an eight-year period from the errors of alcohol and drug-impaired employees. That information, combined with the difficulty of determining individual responsibility in the chaotic aftermath of a major train accident, could have led the Court to allow a presumption of reasonable cause to test all crew members. Instead, the majority in Skinner abandoned entirely any requirement of individualized suspicion and in its place imposed a balancing test that measures the employees' privacy interests against the government's interest in drug testing. Performing this balancing, the Court termed the privacy interests,

those very interests that were significant enough to trigger the Fourth Amendment in the first place, "minimal," while the government's interest in "regulating the conduct of railroad employees to ensure safety", 109 S. ct. at 1414, was "compelling."

The Court also rejected arguments that post-accident testing is unreasonable because the results of blood and urine tests for drugs other than alcohol do not reveal current intoxication or degree of impairment. The Court reasoned that the tests do provide relevant information that may aid investigation, and, in any event, another of the government's purposes is to deter drug use in the first place, and that purpose is furthered by tests that reveal only past use. Consistent with this rationale, a few days after Skinner and von Raab, the Court vacated a decision that the District of Columbia school system could conduct drug tests of certain employees as part of a regular medical exam only if the tests were capable of detecting current impairment. Jones v. McKenzie. 833 F.2d 335 (D.C. Cir. 1987), vacated sub nom. Jenkins v. Jones. 109 S. Ct. 1633 (1989).

The key to the distorted reasoning in Skinner is its companion case, von Raab, which could not have been decided as it was under pre-Skinner Fourth Amendment analysis. There was no evidence of a drug problem in the Customs Service. In his announcement of the program to employees, the Commissioner of the Service described the testing program as setting "an important example in our country's struggle with this most serious threat to our national health and security," 109 S. Ct. at 1401; as the dissent pointed out, this is pure symbolism. Of the first 3,600 employees tested, there were only 5 positive results. Clearly, there was no basis for suspicion of any individual or for the kind of group suspicion that might have been found in Skinner. Having adopted a new balancing test in Skinner, however, the majority found several compelling governmental interests in drug testing: "ensuring that front-line interdiction personnel are physically fit, and have unimpeachable integrity and judgment," 109 S. Ct. at 1393, eliminating "the risk that employees who may suffer from impaired perception and judgment will be promoted to positions where they may need to employ deadly force," id., "deterring drug users from seeking such promotions," id. at 1395, and "protecting truly sensitive information from those who, under compulsion of circumstances or for other reasons, . . . might compromise [it]," id. at 1396. It was on this last point, protecting important information, that the remand occurred. The Customs Service had included in its testing program positions such as "Accountant," "Animal Caretaker," Baggage Clerk," "Attorney," and "Messenger." It was not clear from the record that all occupants of these positions would actually have access to classified information.

Attorney General Thornburgh has declared that Skinner and von Raab give a "green light" to drug testing, in both the public and private sectors, and in large part, he may be right. Among the questions that remain to be answered in the wake of these

decisions is what other groups of workers may be subjected to suspicionless drug testing. Although there have been arguments that safety is the only justification for testing, the Court relied on both public safety and integrity concerns. The safety rationale certainly would include all law enforcement and most other public safety officers, as well as the operators and maintainers of means of mass transportation. In addition, both majority opinions and Justice Scalia's dissent in von Raab mentioned favorably a Court of Appeals decision, Rushton v. Nebraska Public Power District. 844 F.2d 562 (8th Cir. 1988), which upheld testing of workers at a nuclear power plant. As Justice Scalia pointed out in his dissent in von Raab, the safety rationale would also include the operators of dangerous equipment, construction workers, and school crossing guards, as well as automobile drivers. Cf. National Gypsum Co. v. Kansas Employment Security Board of Review. 244 Kan. 678, 685, 772 P.2d 786 (1989) ("The threat posed by employee drug use ... is particularly dangerous when employees are engaged in the operation of heavy equipment, work on wallboard production lines, and use extremely hazardous materials. . . .")

The Court's second rationale for drug testing was the need to insure that employees could not be bribed or blackmailed into revealing sensitive information or compromising the mission of their agency because of a drug habit. This integrity/honesty factor certainly justifies testing members of the military and employees of law enforcement and intelligence agencies. The Court's remand on the question of access to classified information does indicate that there must be some people working in this country who may not be subjected to suspicionless drug testing, but a powerful argument can be advanced that the Court's reasoning might include others with access to important, although perhaps not "classified," information. For instance, employees with knowledge of trade secrets, or employees of law firms, accounting firms, and publicly-traded corporations with access to "insider" information, might be equally subject to blackmail or bribery.

Another major unanswered question from Skinner and von Raab is the constitutionality of random testing. A few lower courts have allowed random testing in what they termed "pervasively regulated" industries, on the ground that workers in those fields have a reduced expectation of privacy. See, e.g.. Shoemaker v. Handel. 795 F.2d 1136 (3d Cir.), cert. denied. 479 U.S. 986 (1986) (jockeys) ; Rushton v. Nebraska Public Power District supra (nuclear power plant workers); Policeman's Benevolent Association v. Township of Washington. 850 F.2d 133 (3d Cir. 1988), cert. denied. 109 S. Ct. 1637 (1989) (police officers); McDonell v. Hunter. 809 F.2d 1302 (8th Cir. 1987) (prison employees). On the other hand, district courts have been routinely striking down the random testing components of Executive agency testing programs. See, e.g., Bangert v. Hodel, 705 F. Supp. 643 (D.D.C. 1989) (random testing of Department of Interior workers in "sensitive" positions unconstitutional); NTEU v. Lyng, 706 F. Supp. 934 (D.D.C. 1988) (computer specialists and motor vehicle operators in Department

of Agriculture); Harmon v. Meese, 690 F. Supp. 65 (D.D.C. 1988) (Department of Justice employees). If "random" means only "suspicionless", random testing entails a great potential for supervisory abuse and harassment. A truly random system, however, such as one in which individuals to be tested are selected by lottery, would eliminate the supervisor's discretion and thus the possibility of discriminatory selection. One of the reasons no warrant was required in Skinner was the unlikelihood of abuse of discretion by railroad supervisors; similarly, in von Raab the occasions for testing were set forth in the regulations and were not controlled on an individual basis by Customs Service officials. Further, one of the compelling governmental interests found in both cases was deterring drug use. If one is interested in deterring workers from ever using drugs, a truly random testing program would be a very effective mechanism.

Handicap Discrimination

A "predictable. and inevitable consequence" of employment actions based on the results of drug tests will be challenges under federal and state laws prohibiting discrimination on the basis of handicap, although those challenges may require employees to make some fairly unsavory arguments about themselves. The Rehabilitation Act of 1973 prohibits Federal contractors and recipients of Federal financial aid from discriminating against "otherwise qualified individuals with handicaps." 29 U.S.C. §§ 793(a), 794. For employment purposes, an "individual with handicaps" is defined as a person who has or is perceived as having "a physical or mental impairment which substantially limits one or more of such person's major life activities." 29 U.S.C. § 706(8) (B). Almost all of the states have laws prohibiting discrimination against the handicapped; their coverage varies, but most follow the federal definition. While alcoholism and drug addiction have been found to be impairments, a 1978 amendment to the Rehabilitation Act excludes from its coverage for employment purposes "any individual who is an alcoholic or drug abuser whose current use of alcohol or drugs prevents such individual from performing the duties of the job in question or whose employment, by reason of such current alcohol or drug abuse, would constitute a direct threat to property or the safety of others." 29 U.S.C. § 706(8) (B). The Supreme Court has commented that by this amendment Congress did not intend to exclude from the protection of the Act all alcoholics and drug addicts. School Board of Nassau County v. Arline, 107 S. Ct. 1123, 1130 n.14 (1987). Therefore, the issue will be the significance of a positive test. Would a positive test for marijuana, for instance, allow an employee to argue that he or she is a "drug abuser" and therefore protected from discharge unless his or her drug abuse constitutes a direct threat to property or safety? Casual use of drugs, particularly alcohol, might be found to be either not an impairment at all, or at the most a "voluntary" impairment, which is probably not covered by the Act. On the other hand, any consumption of illegal drugs might be considered "abuse," or an employee who tested positive

might contend that he or she is in fact an abuser. To date, there have been only two reported cases on this point, although more litigation can be anticipated as the threshold questions of constitutionality are answered. In Burka v. New York City Transit Authority. 680 F. Supp. 590 (S.D.N.Y. 1988), the Rehabilitation Act was held to apply only to those otherwise qualified drug abusers who have been or are being rehabilitated, and not to individuals who tested positive but had not sought rehabilitation. In McCleod v. City of Detroit. 39 Fair Emp. Prac. (BNA) 225 (E.D.Mich. 1985), the court assumed that use of marijuana is a "physical impairment," but held that being denied a job as a fire fighter (the position at issue) did not "substantially limit. . .[the] major life activity" of working; there were still plenty of jobs in the labor market for which plaintiffs were qualified. If drug testing of applicants becomes commonplace, this rationale may no longer suffice, for continuing positive tests may make an individual unemployable.

STATE LAW

State Constitutional and Statutory Rights

In a few States, constitutional protections against unreasonable searches and seizures or invasions of privacy have been interpreted more broadly than their federal counterparts. See Horsemen's Benevolent and Protective Association. Inc. v. State Racing Commission. 403 Mass. 692, 532 N.E.2d 644 (1989) (random and reasonable suspicion drug testing of licensees in state racing industry violates the Massachusetts Declaration of Rights); Hennessey v. Coastal Eagle Point Oil Co., No. W-003611-86 (N.J. Super. Ct. 4/28/89) (random drug testing of private sector employees violates New Jersey constitution). Over half the States have considered legislation authorizing or regulating drug testing; as of this writing, twelve, Iowa, Kansas, Louisiana, Minnesota, Nebraska, North Carolina, Rhode Island, Tennessee, Utah, Vermont, Virginia, and Wisconsin, have enacted laws on the subject. While most of these laws restrict an employer's freedom to test and limit disciplinary action after a positive test, the Utah statute gives great freedom to private sector employers and limits employee rights. Skinner held that when a private search is the result of the Government's "encouragement, endorsement, and participation," 109 S. Ct. at 1412, the Fourth Amendment is implicated. Federal regulations requiring private sector employers to conduct drug tests will probably be held to preempt conflicting State laws. See French v. Pan Am Express. Inc., 869 F.2d 1 (1st Cir. 1989) (Rhode Island drug testing statute is preempted by Federal Aviation Act, as applied to a pilot for an interstate air carrier); Alverado v. Washington Public Power Supply System. 111 Wash.2d 424, 759 P.2d 427 (1988), cert. denied. 109 S. Ct. 1637 (1989) (Federal preemption of the field of nuclear safety prevents application of Washington Constitution to drug testing at nuclear power plant).

Common Law Torts

State tort law offers a variety of possible challenges to drug testing, such as wrongful discharge, invasion of privacy, outrage, defamation, and negligence. There has been very little litigation under State common law to date, but it can be anticipated that State courts will be heavily influenced by developments under the Fourth Amendment, and employers with well-designed programs containing sufficient procedural safeguards will not face a great risk of tort liability. Even though most States have recognized exceptions to the employment-at-will rule, workers fired for failing a drug test must find some public policy embodied in State law that his or her firing allegedly violated. In States with drug testing statutes, a firing in compliance with the law will not be found to violate public policy. Even in States without statutes, it may be hard for at least some workers to make a compelling public policy argument, see Luedtke v. Nabors Alaska Drilling Inc., 768 P.2d 1123,4 Indiv. Emp. Rights Cases (BNA) 129 (Alaska 1989) (public policy supporting health and safety outweighs public policy supporting employee privacy rights; discharge of two workers on North Slope oil drilling rigs who tested positive for marijuana upheld). Moreover, since there is no cause of action for "wrongful refusal to hire," applicants who fail pre-employment drug tests are cut of luck.

torts of outrage, such as invasion of privacy and intentional infliction of emotional distress, require egregious behavior, "an unjustified intrusion ... of such magnitude as to cause an ordinary individual to feel severely offended, humiliated, or outraged." K-Mart Corp. Store No. 7441 v. Trotti, 677 S.W.2d 632, 636 (Tex. App. 1984) . Once again, well-designed drug testing programs that conform to public sector constitutional requirements, where employees are given advance notice of the testing program, and where reliable tests are used, will probably stand up to most tort challenges. See, e.g., Trotti (invasion of privacy to search employee's locker, where employees were allowed to bring their own locks); Kelley v. Schlumberger Technology Corp., 849 F.2d 41 (1st Cir. 1988) (affirming verdict against employer for invasion of privacy and negligent infliction of emotional distress under Louisiana law, where employee was fired for failing a drug test that involved direct observation of urination); Bodewig v. K-Mart, Inc., 54 Or.App. 480, 635 P.2d 657 (1981), pet. denied. 292 Or. 450, 644 P.2d 1128 (1982) (tort of outrageous conduct found where employer required strip search of employee suspected of theft, in presence of complaining customer).

AUTHOR

Elinor P. Schroeder, J.D.
Professor of Law
University of Kansas School of Law
Lawrence, Kansas 66045

Drug Screening in the Workplace: Use, Abuse and Implications

John Grabowski and Peter B. Silverman

INTRODUCTION

Drug abuse constitutes a complex and substantial problem for which numerous solutions have been proposed. Typically, the goal has been to produce rapid and dramatic change. This has not been achieved at any of the possible points of intervention. Walsh (1989) has noted that efforts to reduce supply at the source, or by capture of supplies in transit have been of limited effectiveness. Reports of the effectiveness of prevention efforts to reduce demand are equivocal. The "zero tolerance" approach with users has limitations. Finally, the development of efficacious treatments has been a slow and difficult process.

The perceived magnitude of the problem and frustration with the results of each of the single interventions (optimally implemented or not) have led to proposal of exceptional solutions. They are exceptional in so far as they stray substantially from standard practice, require substantial effort for the possible benefit, or are contrary to the current wisdom or indications of available data. Thus, for example, at one extreme, Buckley (New York Times), proposed that all drugs should be legalized. At the other extreme, the concept of "zero tolerance" emerged with the view that any contact with drugs should bring rapid and harsh penalties. Concurrently there has emerged the call for panpopulation urine screening. This has been variously proposed as an adjunct, a deterrent, or a cure.

The desirability of the goal of reducing drug abuse in the workplace is clear. The means for doing so are less clear. Fundamental concerns reside in: whether drug screening serves any of the proposed functions; the determinants of its efficacy; and the possible short and long term consequences.

PROCEDURES AND CIRCUMSTANCES FOR DRUG SCREENING

A. Drug Testing Technologies, Procedures, and Costs

The technologies for screening, their limitations and advantages have been discussed extensively in the literature and summarized by Hawks and Chiang (1987). Strict adherence to chain of custody rules for specimens can reduce the probability of errors from this source, although they can never be eliminated. Appropriate combinations of immunoassays, chromatographic and spectrographic techniques can provide reliable screening and confirmation procedures. Precise laboratory procedures potentially assure a specified level of certainty concerning results. Yet problems of poor quality control will be difficult to resolve in the public sector. As Finkle has noted, "In the climate where there is money to be made, inevitably there will be incompetent and inadequately staffed laboratories... the tests are very easy to do badly and difficult to do well" (NYT, 1986). Elaborate and costly efforts to increase quality control being implemented by various professional societies and the NIDA can be expected to reduce, but not eliminate, this source of error. In brief, under the best of conditions, the tests can produce reliable results but problems of unreliability can emerge under conditions that are presumed to be acceptable (Lundberg, 1986; Hansen et al, 1985).

While costs for testing with high specificity and sensitivity are substantial, it is likely that these costs can be reduced by improvements in technology and increased volume of testing. However, the current costs of drug testing by proficient laboratories using appropriate techniques have been estimated by Miike (personal communication, 1988) and are considerable. High volume and the special circumstances for the military testing program minimized costs, yet 3.6 million drug tests with 92,653 positives, cost the military over $52 million in 1986 (Miike, personal connnunication, 1988).

B. Types of Screening

The Urban Mass Transportation Administration (UMTA) has described the range of possible employment testing strategies in nonclinical settings related to transportation workers as imposed on December 21, 1988 (53 FR 47156; 1988). These requirements have had the most pervasive reach outside of the military screening program. The basic types of testing specified include; preemployment screening, random screening during employment, reasonable cause screening, return to duty screening, and post accident screening. (An additional category in part intended to preclude performance enhancement has been incorporated in athletics). The goal of the several categories is said to be "a drug free workplace". While several of these approaches are preventive, others are intended to place blame.

Preemployment screening assures that only those who have abstained from drug use for a sufficient period will be

employed. Random screening will identify those using behaviorally active drugs and is intended to detect and have deterrent value. However, it appears that most random testing is insufficiently frequent to have demonstrable benefit in terms of prevention. For example, following the 50% rule, employees are tested only once every two years. Patterns of drug use can alter dramatically during such extended periods. Further, some companies refrain from random screening because of the potential for legal action. This view was voiced at DOT hearings by those in the transportation industry. Procedural problems also exist such as keeping testing dates confidential and the need for elaborate bookkeeping and supervision.

Reasonable cause screening can be viewed as quasi-diagnostic. If an employee has been observed to engage in inappropriate behavior, testing may be requested by supervisors. (Two potential problems are that a minimally trained supervisor is making judgements about drug effects and performance in a situation rife with conflicts, and the behavioral problem itself may warrant referral to an Employee Assistance Program whether or not its origins reside in drug abuse). Return to duty screening with long term repeated follow-ups is typically related to prior positive screens but may also be used as a means of implementing screening on existing employees not covered by preemployment screening. Finally, post-accident testing is explicity intended to identify or rule out factors contributing to accidents. In general, screening other than post-accident is an effort to preclude impaired performance. Testing in athletics on the other hand addresses drug related enhancement of performance, may deter drug use that might permit continued participation despite injury, and presumably is intended to have some larger social benefit.

Reasonable cause testing and particularly the subset of post-accident testing may be the most widely accepted types. Currently, preemployment screening is the most commonly used.

C. Screening Sites and Poulations

The general acceptability of screening appears to be related to perceived consequences of drug effects on performance. However, it is also determined by factors such as company size, union strength, and cost. Large companies are more likely to screen than small companies. Cost of testing programs, insurance, presence of EAPS, and treatment are pertinent in these decisions. Schools may be thought of as special workplace sites and whether or not testing can or will be implemented depends on the community view. Ironically, one school district that imposed testing did so for all drugs other than alcohol and tobacco (NYT, 1986) thereby following the approach of most industrial testing programs.

There is no agreement on who should be tested. "Fairness" might dictate that all, if any, employees should be tested and some companies have taken that approach. Others acknowlege

privately that differential rules apply. Data on drug abuse (e.g. Cook, 1988) suggest that some subgroups of the work force have higher prevalence rates and therefore a data based approach would adhere to differential, or targeted populations for testing. Others have suggested that testing should focus on professions based on risk in lives, money, or power. Data strongly support screening during treatment for drug abuse, and the high correlations between drug/alcohol use and accidents dictate that post accident testing is indicated. (Peculiarly, some treatment programs refuse to test due to concerns that patients will feel they "are not trusted.") However, the issue of who to test has not otherwise been satisfactorily addressed.

D. Policies

It has been stated that the "model policy" is intended to "get the substance abusing employee into treatment, afford the opportunity for help, and get the individual back on the job" (Walsh, 1989). There are no data about the extent of adherence to the model policy. It can be expected that small companies have difficulty implementing this costly approach. Policies can be dramatically changed by events. Thus for example, Exxon did not use available data in terms of fitness for duty for a tanker captain and has now announced a policy that assures dismissal of those found to have used drugs. AMTRAK, like Exxon, had not used previously available data concerning those involved in a serious accident. Punitive rather than rehabilitative policies are likely to be the mode given the costs of the latter.

CONSEQUENCES OF TESTING

A. Reduction in Illicit Drug Use

Proponents of drug screening in the work place predict the consequence will be reduction in drug use in this environment. They further believe that other drug use will be reduced. There are some data such as those from the military that suggest reductions may occur. However, screening in that highly regulated environment is not an adequate test of the effect. Evaluation of changes in rate of positives due to the screening program in civilian environments will necessarily be contaminated. Substantial public interest, concern, and the changing perceptions about drug use have apparently produced the reductions that Musto (1987) predicts in his description of cycles of cocaine use (e.g. see NIDA, 1989, High School Senior Survey). Reduced rates of drug use by current high school seniors are predictive of reduced rates of use by the next generation of employees. Therefore, future reductions of drug use attributed to screening programs may be in fact the result of other processes. In the face of substantial cost and problems, determination of the contribution of effects of drug screening on drug use should precede widespread implemention. Further, it should be noted that the drug use rates in some environments are sufficiently low (Cook, 1988) to assure that there will be little evidence of effect. It is more likely that screening programs in low use environments will create

problems for nonusers, given false positives rates.

B. Reduction in Therapeutic Drug Use

Grabowski and Lasagna (1987) proposed that a major concern that emerges with the current furor about drug use is exacerbation of lay public fears about being "addicted". Added to this is the new concern that they will be "caught" using their therapeutic agents. Some individuals may stop using therapeutic agents prior to a known drug screen to preclude the need for responding to questions about use or about their health status. While stated policies indicate that providing a list of prescription (or nonprescription drugs) prior to testing will obviate the need for concern, it presents obvious problems. This is an undesirable consequence since compliance is a major problem that can be complicated by drug screening .

C. Public Health Issues

Decreases in substance abuse will enhance the public health. It is unclear that drug screening will have benefit proportional to the cost. The data suggest that drug abuse in industry is relatively low, although high enough to be of concern in certain subgroups. Further, it can be argued that in some groups such as pilots any use is problematic although the FAA acknowledges there are no data to support current concerns (53 FR 47156; 1988). (In fact, the gravest public health problems appear to be in unemployed drug abusing populations.) To the extent that testing patterns in companies do not reflect the "model policy", individuals who are fired due to drug use may resort to more, rather than less, drug use. While the company is no longer responsible, the public sector must deal with a more severe problem with few constraints on the individuals behavior. Despite the need to resolve the potentially severe problems in the workplace, resolution through dismissal has difficulties parallel to those of expulsion of young people from school.

D. Legal Issues

The First legal concerns are constitutional. When the employer is "the state", or one acting for the state (e.g. via contract or grant), the employee has a constitutional right of privacy that cannot be encroached on in the absence of compelling interest. The private employer with no link to the state has substantial freedom to determine conditions of employment. Commenting on a specific case involving random unannounced testing of firefighters, Judge H. Lee Sarokin, noted that "the harassment, coercion, and tactics utilized here, even if motivated by the best of intentions should cause us all to recognize the realities of government excesses and the need for constant vigilance against intrusions in constitutional rights by its agents"(NYT, 1986). Judge Sarokins point reflects dissatisfaction with the necessarily intrusive character of testing if it is to achieve the goal of reducing or deterring drug use; i.e. properly implemented it will be intrusive. A critical concern is whether decisions on panpopulation drug

screening will provide the precedent of screening for other disease or behavioral problems including those with genetic origins whether or not relevant to performance.

Secondary legal issues include tort liability of the employer for the acts of its employees. Is the company that does not test or intervene liable if its employees under the influence of drugs cause an accident, cost fortunes in the stock market, or otherwise harm the public. Certainly, dram shop laws place responsibility on bars for serving liquor to intoxicated individuals subsequently involved in accidents. Corporations may be held liable for accidents whether or not drugs were contributory and thus have a vested interest in decreasing all determinants over which they have control. Many corporations, including the transit authorities now subject to the UMTA regulations, are concerned they might be held liable for untoward consequences of random testing. For example, an employee fired due to a false positive could sue with costly consequences.

RELATIONSHIPS TO OTHER TESTS OR CONTINGENCIES

There is no question that employers, educators, and directors of organizations must have contingencies and consequences to govern behavior. Absences as well as inappropriate behavior on the work premises are of interest to the employer. The questioned relationships are those between positive urines, behavior and consequences. Similarly one should question denial of employment based on HIV positivity rather than inability to complete work. Abberant behaviors about which employers are concerned are observable without drug screening and have numerous possible causes other than drug use.

Wide spread screening for drugs may be the precursor for pervasive HIV or other screening. Genetic screening is already in place in some employment settings although it has been observed to be unjustified given available data (Murray, 1986). Future genetic testing will face similar issues with progress of the Genome Project mapping the human genetic makeup. Expert concerns have already led to contesting acceptance of certain procedures as evidence in courts (Lewin, 1989). To the extent that drug screening sets precedents for employee-employer or population - government relationships, great caution should be used in implementation.

CONCLUSIONS

Several questions were posed at the outset. The answer to the question of whether screening can serve as preventive, cure, or adjunct is circumstance dependent. Screening in treatment settings with proper behavioral contingencies is an essential tool, but must be properly implemented (e.g. Havassy and Hall, 1982). There is little evidence that random testing procedures as proposed will have the desired effect. The main preventive consequence of testing will be that those who can not abstain

for even brief periods will fail preemployment screening.

Current systems for testing will not substantially alter the general problems of drug abuse for several reasons. First, drug abuse is most prevalent in unemployed populations. Second, it occurs at low rates in most industries. Third, the consequences of improperly implemented tests may be more severe than benefit attained. The cost of testing properly is more substantial than most companies can afford and thus model policies will not be implemented. There has not been suitable determination of the long term consequences in relation to broader social and technological issues or on the prevalence of drug use itself.

Systematic studies of, screening and outcomes in industry will be the only means for evaluating the real consequences of testing programs. These studies must meet all criteria for clinical studies or they will be intolerably confounded by the changing social circumstances. The American Society for Clinical Pharmacology and Therapeutics has called for research in diverse areas related to screening. This must be paralleled by thoughtful study of the related social issues with heavy reliance on the data. Most important, a critical evaluation of the range of issues and consequences should be addressed in a scientific forum.

REFERENCES may be obtained from the authors.

ACKNOWLEDGEMENTS: The authors wish to thank their colleague Dr. Richard Meisch for his thoughtful comments on earlier iterations of this paper.

Authors
John Grabowski, Ph.D. and Peter 8. Silverman, Ph.D., J.D.
Substance Abuse Research Center
Department of Psychiatry and Behavioral Sciences
University of Texas Health Science Center
1300 Moursund
Houston, Texas 77030

Neurobehavioral Teratogenicity of Gestational Cocaine Exposure

Linda Patia Spear, Cheryl L. Kirstein, Nancy A. Frambes and Carole A. Moody

An escalating number of human offspring are born to mothers who have used cocaine during pregnancy; as neonates these offspring appear to exhibit a number of behavioral and physiological alterations (e.g., Chasnoff et al., 1987). In view of the increasing need for the establishment of animal models to assess the effects of early cocaine exposure, a number of research groups including our laboratory have begun to investigate the neurobehavioral consequences of gestational exposure to the dopamine (DA) uptake inhibitor cocaine. In our work, we have focused initially on assessment during the early postnatal period. Given the available clinical population of exposed offspring, correlations between human and animal data might be expected to be closer when examining laboratory animal populations early in life. We have observed that prenatal cocaine exposure results in a number of behavioral, biochemical and physiological alterations in the offspring when examined early in life, as summarized below.

GENERAL DOSING AND TREATMENT PROCEDURES

In these experiments, Sprague-Dawley rat dams were subcutaneously (s.c.) injected during the mid-light cycle with cocaine hydrochloride daily from gestational days 8-20. (E8-20) at doses of 0 (saline control) 10, 20 or 40 mg/kg/3cc. In-some of our work, we have focused only on the 40 mg/kg dose, a dose well above threshold for producing neurobehavioral alterations. Control dams included in these experiments consisted of dams allowed ad lib access to lab chow (LC), and in some studies dams pair-fed to animals in the 40 mg/kg dose group (PF). In all but our initial study (Spearu et al., 1989b), all offspring were fostered to surrogate dams on postnatal day 1 (P1).

COCAINE/BENZOYLECGONINE (BE) DATA

To determine whether s.c. administration is an appropriate method for administering cocaine to the dams, we examined the distribution of cocaine-and the cocaine metabolite benzoylecgonine (BE) in brain and plasma of dams and their near-term fetuses 0.5 and 2 hrs. post-injection on E20 following chronic daily s.c. injections of 10, 20 or 40 mg/kg/3cc cocaine beginning on E8 (Spear et al., 1989a). Dose-dependent increases in brain and plasma levels of cocaine were observed in both the dams and fetuses. Maternal plasma levels were found to be in the range of, or exceeding, those reported in human cocaine users. Significant amounts of both cocaine and BE were observed in fetal brain. Taken together, these results suggest that the s.c. route is appropriate in rats for administering cocaine in investigations of potential neurobehavioral ramifications of gestational cocaine exposure.

MATERNAL/LITTER DATA

In general, these doses of cocaine produced few notable effects on the progress of gestation or fetal physical development. In a number of our studies maternal weight gain was observed to be slightly reduced during pregnancy (by 6-9 percent) in dams treated with 40 mg/kg cocaine. However, no significant difference in offspring body weights at either P1 or P21 were found in cocaine exposed offspring in any of our studies. No differences in gestational length or in resorption rates were observed, athough non-significant trends for an increase in resorption rate was sometimes noted with the 40 mg/kg dose. Moreover, no differences were observed in litter size or the ratio of male/female pups in the litters. Cocaine-exposed offspring also exhibited normal reflex maturation and development of physical landmarks (see Spear et al., 1989b).

COGNITIVE TESTS

In spite of normal rates of physical development, offspring exposed gestationally to cocaine exhibit pronounced cognitive deficits when tested during the neonatal to weanling age periods. Cocaine-exposed offspring often fail to exhibit evidence of conditioning even in simple classical conditioning tasks that are readily learned by control age-mates, particularly in circumstances when the training parameters used produced only modest levels of conditioning in control offspring (see Spear et al., 1989c, for further discussion). In more challenging cognitive tasks, these cognitive deficits may be particularly pronounced. In future work, we plan to investigate in more detail the circumstances under which-cocaine-exposed offspring exhibit these learning deficits, and to examine whether the deficits in cognitive performance observed during the neonatal to weanling age period are also observed later in life.

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BEHAVIORAL AND PSYCHOPHARMACOLOGICAL APPROACHES TO EXAMINE DA ACTIVITY

Behaviorally, cocaine-exposed offspring exhibited a reduction in footshock-precipitated wall climbing at P12 in the absence of alterations in footshock sensitivity thresholds (Spear et al., 1989c). Given that wall climbing has been previously shown to be strongly related to levels of catecholamine activity at this age, these data suggest that there may be some net attenuation in catecholaminergic function in pups exposed gestationally to cocaine. Cocaine-exposed offspring also exhibit an attenuated response to the DA agonists apomorphine (P12 test) and cocaine (P11 test), along with an accentuated response to the DA antagonist haloperidol (P8 test) (see Spear et al., 1989c). This pattern of psychopharmacological alterations is consistent with the suggestion that there may be a functional attenuation in DA activity in these offspring, a hypothesis that needs to be tested using a multitude of approaches as discussed further below.

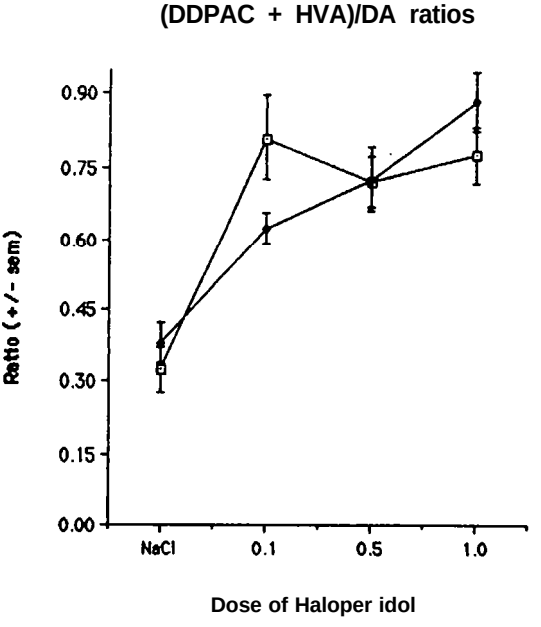

Figure 1 *Ratios of (DOPAC + HVA)/DA following saline, 0.1, 0.5 or 1.0 mg/kg haloperidol in P11 offspring of dams exposed gestationally to 40 mg/kg cocaine (C40) and control dams (LC). Data are collapsed over striatal and nucleus accumbens brain samples.*

BIOCHEMICAL STUDIES OF DA FUNCTION

Because of these behavioral and psychopharmacological signs of potential alterations in the DA system in cocaine-exposed offspring, we have begun to examine DA system function using neurochemical and hormonal response measures. One approach that we have used is to examine haloperidol-induced increases in the ratio of the DA metabolites (DOPAC + HVA)/DA in cocaine-exposed offspring. In this study, DOPAC, HVA and DA were analyzed via HPLC-EC in caudate and nucleus accumbens of P11 rat pups sacrificed 60 min. following injection with saline, 0.1, 0.5 or 1.0 mg/kg haloperidol. The results of this study are shown in Figure 1 which presents data collapsed across nucleus accumbens and striatum as there were no significant interactions involving brain region in our analyses of these data. In these P11 pups, as is typically the case in adults, haloperidol increased (DOPAC + HVA)/DA ratios. However, offspring exposed gestationally to cocaine showed significantly less of an increase in this ratio in response to the low (0.1 mg/kg) dose of haloperidol than LC control pups.

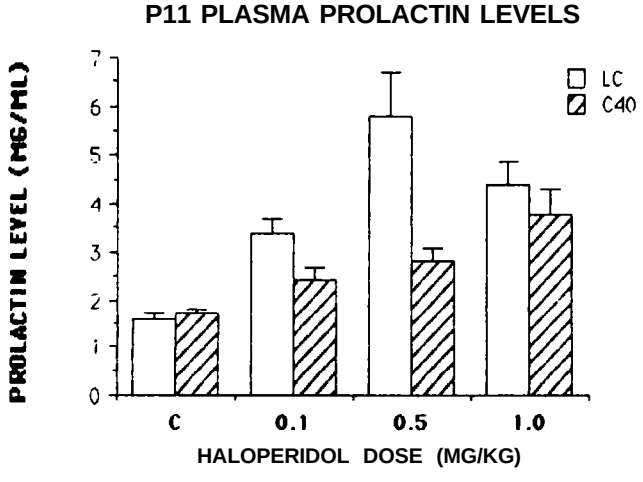

Figure 2 ***Plasma levels of prolactin in P11 offspring of dams given 40 mg/kg cocaine (C40) or injected with saline and given free access to food (LC), with the pups being sacrificed either immediately upon removal from the nest (C) or 60 min. following 0.1, 0.5 or 1.0 mg/kg haloperidol.***

In collaboration with Dr. Cynthia Kuhn (Duke University) we have also examined DA in the tuberoinfundibular system where DA exerts a chronic inhibitory effect on prolactin secretion. Treatment with the DA antagonist halopetidol increases prolactin release by blocking this inhibition. As can be seen in figure 2, cocaine-exposed offspring exhibited a reduction in the magnitude of the haloperidol-induced increase in prolactin at the two lower doses of haloperidol. These data suggest that the tuberoinfundibular DA system, like striatal and mesolimbic DA systems, may be sensitive to the effects of gestational cocaine exposure.

These attenuated neurochemical and hormonal respones to low doses of halopendol in offspring exposed gestationally to cocaine are in contrast to the psychopharmacological data discussed above where cocaine-exposed offspring were observed to be more sensitive to halopetidol-induced behavioral alterations. Although a number of hypotheses can be generated as to how these findings can be reconciled, without additional data it may be premature to do so. Gestational exposure to cocaine may induce a multitude of alterations within the DA system that may ultimately affect DA function. Some of these alterations may be directly induced by the drug exposure per se, whereas others may be compensatory to initial drug-induced alterations in the development of portions of this system. Consequently, it may not be appropriate to infer from data limited to but a few indices of a neurotransmitter system as to how the functioning of that neurotransmitter system as a whole is altered by gestational drug exposure. Thus, although these data suggest that exposure to cocaine early in life alters subsequent functioning within the DA system, other aspects of the DA system need to be examined (D1 and D2 receptor binding, density of presynaptic DA terminal endings, number of DA uptake sites, etc.) to derive a profile of alterations in the DA system necessary for determining the ultimate impact of gestational cocaine treatment on DA function.

OTHER NEUROCHEMICAL AND PHYSIOLOGICAL ALTERATIONS

Neurochemical alterations in cocaine-exposed offspring are not restricted to the DA system. In work conducted in collaboration with Dr. Ken Leskawa (University of Louisville), we observed that offspring exposed gestationally to 40 mg/kg cocaine exhibited a transient elevation in whole brain levels of gangliosides and glywlipids evident at P1 but not P11. These substances play a number of critical roles in development, including cell-cell recognition, cell surface structural changes and regulation of protein receptor function and cell growth. Given that levels of particular gangliosides and glywlipids show developmental increases, whereas others decline, the significance of these transient elevations in gangliosides and glywlipids in cocaine-exposed offspring remain to be determined.

Cocaine-induced alterations in offspring may not be restricted to the nervous system. We have preliminary data on thymus weights of pups exposed to 40 mg/kg cocaine during gestation and control pups. As can be see in figure 3, cocaine-exposed pups exhibited a transient decrease in thymus weights at P1 which was no longer evident by P11; conversely, a significant increase in thymus weights (compensatory hypertrophy?) was seen in cocaine-exposed offspring at P21. These preliminary data suggest a potential alteration in immune system function in offspring exposed gestationally to cocaine, a possibility that needs to be examined further.

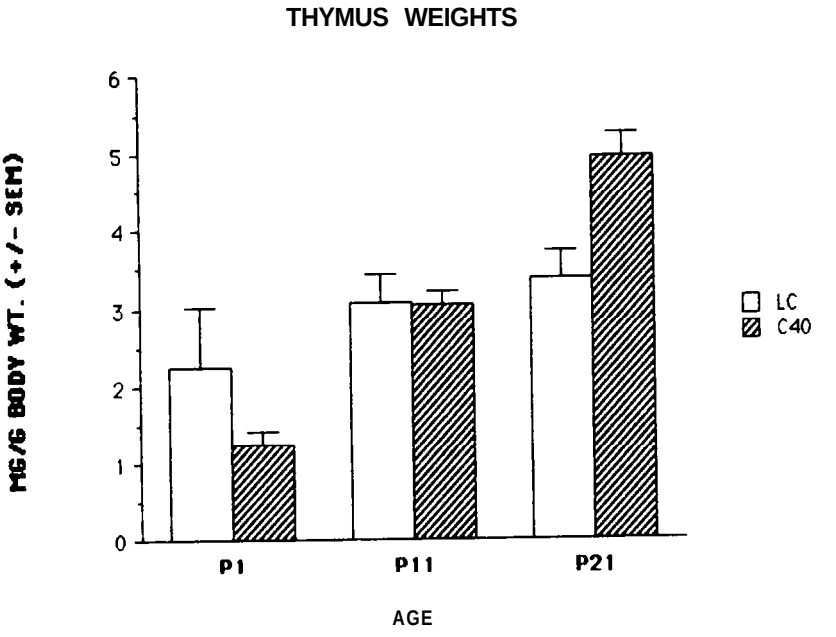

Figure 3 ***Thymus weights of P1, P11 and P21 offspring of dams given 40 mg/kg cocaine (C40) or injected with saline and given free access to food (LC).***

SUMMARY

Gestational cocaine exposure has an impact on a number of behavioral and physiological measures examined during the neonatal to weaning age period. Notable effects of early cocaine exposure include a disruption in cognitive function, along with a profile of DA alterations. Observed physiological differences do not appear to be restricted, however, to the DA system, or even the nervous system. Such data may have important implications regarding the prognosis for future development of human offspring exposed gestationally to cocaine.

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ACKNOWLEDGEMENTS

Drs. Cynthia Kuhn and Kenneth Leskawa collaborated with us on the hormone and ganglioside/glycolipid experiments, respectively. This research was supported by National Institute of Drug Abuse grant R01 DA04478.

AUTHORS

Linda Patia Spear (Ph.D.), Cheryl L. Kirstein (M.S.), Nancy A. Frambes (B.S.) and Carole A. Moody (B.S.)
Department of Psychology and Centers for Developmental Psychobiology and Neurobehavioral Sciences
SUNY-Binghamton
Binghamton, NY 13901 USA

Cocaine Binding Sites Related to Drug Self-Administration

Mary C. Ritz, John W. Boja, Frank R. George and Michael J. Kuhar

Cocaine is a powerfully reinforcing drug that leads to several physiological, psychological, and sociological problems in users. It produces this and a variety of other pharmacological effects through its interactions with several central nervous system binding sites. It is well established that cocaine inhibits monoamine uptake, and that these transporters appear to be labelled by ^{3}H-cocaine (Reith et al., 1980; Kennedy and Hanbauer 1983; Schoemaker et al., 1985; Calligaro and Eldefrawi 1987). In addition. studies from our laboratory have shown that (-)cocaine interacts with both sigma and muscarinic cholinergic receptors in brain (Sharkey et al., 1988a, 1988b). The affinity of cocaine for these latter binding sites is much lower than for monoamine transporters, and we have shown that cocaine binding to these sites is not associated with the reinforcing properties of cocaine. However, cocaine interactions with these sites may become relevant at higher, toxic doses of the drug.

Dopamlne Transporters Mediate Reinforcing Properties of Cocaine

The goal of initial studies was to determine the relative importance of various brain receptors in medlating the reinforcing properties of cocaine. We reasoned that the identification of pharmacologically relevant receptors required that an association exist between the potency of cocaine-related drugs in animal models of substance abuse and their potency at binding sites in the brain. It appeared likely that cocaine would exhibit stereospecificity for relevant receptors such that (-)cocaine would exhibit a higher potency for the receptor of interest than (+)cocaine, just as it does in cocaine self-administration studies. Cocaine should also exhibit a micromolar affinity for the receptor of interest since micromolar blood, and therefore brain, concentrations of cocaine are associated with its euphoric effects in humans (Fischman et al., 1976).

Our results illustrated that the potencies of cocaine-like drugs in self-administration studies correlate with their potencies in lnhibiting ^{3}H-mazindol bindlng to the dopamine transporters in rat striatum, but not with the potency in binding to a large number of other presynaptlc and postsynaptic binding sites (Ritz et al., 1987). These results are consist with many previous studies indicating that brain dopaminergic neuronal systems, particularly meso-limblc pathways, have been implicated in the mechanism of action of cocaine (Goeders and Smith 1983; Martin-Iverson et al., 1986; Wise 1984).

Does Amphetamine Binding to Dopamine Transporters Medlate-its Reinforcing Properties?

Amphetamine and cocaine have often been viewed as belonging to the same pharmacological class of drugs, the psychostimulants, and have been shown to produce similar physiologic, behavioral and subjective effects in humans. However, increasing evidence indicates that amphetamine and cocaine have important differences in pharmacological effects and in underlying molecular mechanisms (McMillen 1983; Langer and Arbilla 1984). In addition, our previous data had shown that amphetamine, relative to cocaine, is more potent in self-administration studies than would have been expected based

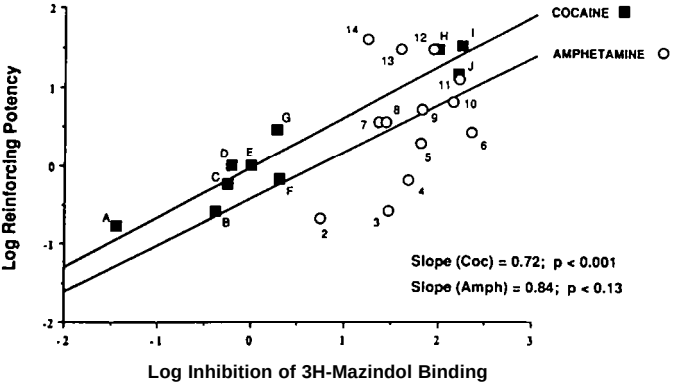

Figure 1: The Bindlng of Cocaine- and Amphetamine-Related Compounds at Dopamine Transporters. Slope and p values were determined by multiple regression analysis: Compounds shown are as follows: A) Mazindol, B) WIN 35,065-2, C) WIN 35,981, D) Methylphenidate. E) 1-Cocaine, F) d,1-Norcocaine, G) Dimethocaine. H) Procaine, I) Chloroprocaine, J) d-Pseudococaine, 2) d-Amphetamine, 3) d,1-Cathinone, 4) 1-Amphetamine, 5) Phentermine, 6) d-Ephedrine, 7) Bentphetamine, 8) Phenmetrazine, 9) Diethylproprion. 10) Phendimetrazine, 11) 1-Ephedrine, 12) Clortemine, 13) d,1-Methylenedioxyamphetamine, 14) Chlorphentermine.

Source: Ritz and Kuhar. J Pharm Exp Ther 248, 1010-1017, 1989.

on its inhibition of mazindol binding at the dopamine transporter. The goal of a subsequent investigation, then, was to determine whether the mechanism of action associated with amphetamine-reinforced behavior might also be associated with dopaminerglc neuronal systems. Thus, we assessed in vitro binding potency and stereospecificity of amphetamine at both pre- and post-synaptic monoamine receptor sites in order to determine which of these sites, if any, might be related pharmacologically to amphetamine self-administration.

The results of this study suggested that amphetamine binding to the mazindol site on the dopamine transporter is not associated with the reinforcing properties of amphetamine, as it was shown to be for the reinforcing properties of cocaine (Figure 1). We found no significant relationship between the reinforcing potencies of phenylethylamines and their inhibition of ^{3}H-mazindol binding into dopamine transporters, and that the potencies of these drugs at this site were not generally predictive of their reinforcing properties. Indeed, our analyses indicates that the reinforcing effects of phenylethylamines were not positively correlated with inhibition of ligand binding to any of the pre- or post-synaptic monoamine sltes tested. However, the self-administration of amphetamine and related compounds appeared to be inversely related to the inhibition of ^{3}H-paroxetine binding to the serotonln transporter, suggestlng that serotonin uptake inhibition opposes the reinforcing effects of amphetamine (Figure 2). This effect does not appear to be related to direct effects of drug binding at 5-HT$_2$ receptor sites (Ritz and Kuhar 1989).

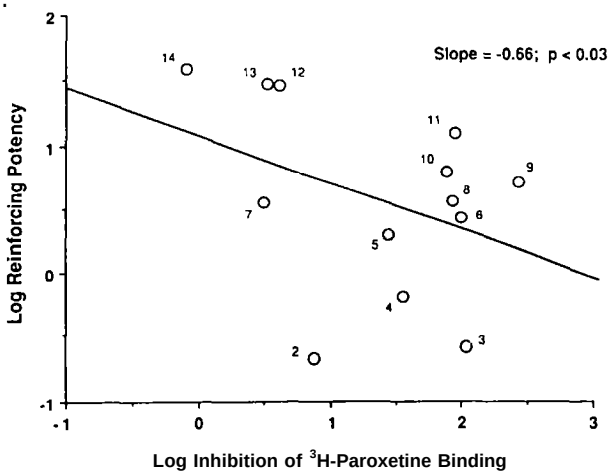

Figure 2: Binding of Amphetamine-Related Compounds at Serotonin Transporters. The compounds shown are named in the legend for Figure 1. Slope and p values were aetermined by multiple regression analysis.

Source: Ritz and Kuhar. J Pharm Exp Ther 248, 1010-1017, 1989.

Our biochemical studies have shown that cocaine, but not amphetamine, binding to mazindol sites on the dopamine transporter is significantly related to the reinforcing properties of these drugs. Furthermore, there appears to be a significant inverse relationship between drug binding to the serotonin transporter and the reinforcing value of amphetamine, but not cocaine. Binding to the norepinephrine transporter sites does not appear to be significantly related to the reinforcing potency of either cocaine or amphetamine.

Serotonerglc Neurons Influence Amphetamine Reinforcement

Behavioral studies of the effects of the potent serotonin reuptake lnhibitor. fluoxetine, on cocaine- and amphetamine-reinforced responding in rats were consistent with the biochemical data described here. Figure 3 indicates that pretreatment with 2.5, 5.0 and 10.0 mg/kg fluoxetine significantly ($p < 0.05$) decreased rates of responding maintained by amphetamine (0.125 mg/kg/inf), but had no effect on responding maintained by cocaine (1.0 mg/kg/inf). Thus, we have obtained evidence from both biochemical and behavioral studies suggesting that the binding of amphetamine and related drugs to the serotonin transporters opposes the reinforcing properties of these drugs. It appears that serotonin uptake inhibition may be important for modulation and attenuation of the rewarding effects of amphetamlne and related compounds (Porrino et al 1989).

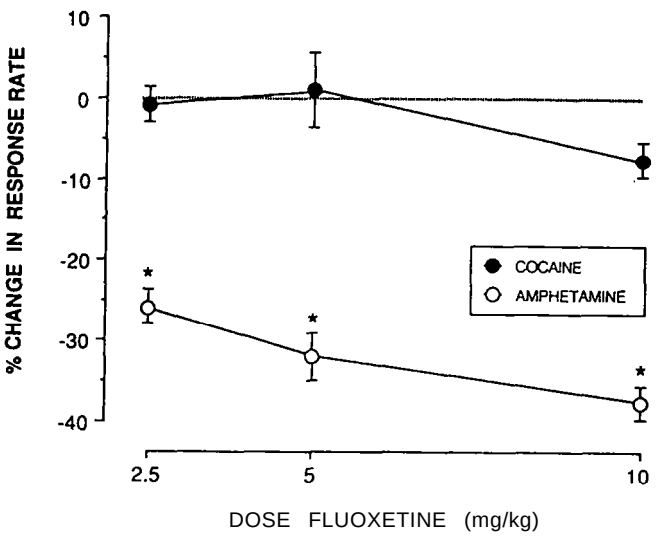

Figure 3: Effect of Serotonin Uptake Inhibition on Cocaine and Amphetamine Self-Administration.

Source: Porrino et al., Life Sci, 1989 (in press).

Does Cocaine Binding to Dopamine Transporters Mediate Genetic Differences in Cocaine-Reinforced Behavior?

Several studies have shown the influence of inherited traits on the reinforcing properties of drugs (George and Goldberg, 1989). Since we have previously shown that the interaction of cocaine with the dopamine transporter was a primary influence on the reinforcing properties of cocaine, we assessed whether the dopamine transporter could account for genetic differences in cocaine reinforcement. Studies have shown genetic differences in the predisposition of several rat strains to ingest cocaine, via the oral route of administration (George and Goldberg, 1989). Cocaine, self-administered orally, served as a strong reinforcer in Lewis rats only. Cocaine served as a marginal reinforcer in NBR rats, and did not serve as a reinforcer in ACI or F344 rats. Receptor binding studies using these same rat strains indicated that there were rio differences in either affinity or number of ^{3}H-GBR 12935 binding sites between any of these four strains. Likewise, there were no differences in either affinity or number of ^{3}H-SCH 23390 and ^{3}H-spiperone binding to D_1 and D_2 receptor sites, respectively. In addition, there were no differences between strains in the affinity of (-)cocaine at ^{3}H-GBR 12935 binding sites on the dopamine transporter.

These results suggest that although the dopamine transporter plays a primary role in determining the reinforcing potency of cocaine in animals for which it serves as a reinforcer, differences in cocaine binding to this site do not account for individual or strain differences in whether or not cocaine serves as a powerful reinforcer. It is possible that further analysis of cocaine binding to dopamine transporters or postsynaptic receptor sites using additional brain regions or autoradiographic techniques would detect significant differences between strains which differ in their propensity to self-administer cocaine. In other studies, autoradiography illustrated significant differences between mouse strains in receptor number (B_{max}) which were undetected or masked using initial receptor binding techniques using relatively crude brain membrane preparations (Belknap 1990, Moskowitz and Goodman 1985).

^{3}H-WIN 35,065-2 Binding to Dopamine Transporters

In a final series of experiments we assessed the binding characteristics of ^{3}H-WIN 35,065-2 to cocaine binding sites on dopamine transporters. In this cocaine analog, the tropane and phenyl rings of cocaine are directly attached to each other, eliminating the ester linkage. This derivative has been shown to serve as a more important reinforcer than cocaine under a fixed interval schedule of i.v. injection (Spealman and Kelleher 1981). It has also been shown that WIN 35,065-2 inhibits ^{3}H-cocaine binding to brain membranes (Sershen *et al.,* 1982). It was hypothesized that this analog of cocaine might prove to have some advantages over cocaine in binding assays because it is more potent in both behavioral and biochemical studies. It is also likely to be more resistant to metabolic degradation in *in vivo* studies do to the elimination of the ester linkage between the benzene and tropane rings.

The results of our study have shown that the binding of ^{3}H-WIN 35,065-2 to striatal membranes is saturable and reveals two binding sites (Ritz et al., submitted). Saturation experiments indicate a low affinity binding site that is apparent K_D of approximately 105 nM and a B_{max} of 115 fm/mg tissue. A high affinity site has also been identified with an apparent K_D of 6.2 nM and a B_{max} of 8.8 fm/mg tissue. The results show that ^{3}H-WIN 35,065-2 binding to both high and low affinity sites in striatum is sodium dependent, and that 6-hydroxydopamine lesions result in a loss of both high and low affinity sites. These results, in addition to pharmacological characterizations, suggest that the ligand binds to sites closely associated with the dopamine transporter. Thus, our results suggest that ^{3}H-WIN 35,065-2. because of the structural similarities to cocaine, its higher binding affinity compared to cocaine and its relatively greater resistance to metabolic breakdown, may be a useful ligand for examining cocaine binding sites on the dopamine transporter.

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AUTHORS

Mary C. Ritz, Ph.D.
Preclinical Branch
NIDA Addiction Research Center
P.O. Box 5180
Baltimore, MD 21224

John W. Boja, Ph.D.
Neuroscience Branch
NIDA Addiction Research Center
P.O. Box 5180
Baltimore, MD 21224

Frank R. George, Ph.D.
Preclinical Branch
NIDA Addiction Research Center
P.O. Box 5180
Baltimore, MD 21224

Michael J. Kuhar, Ph.D.
Neuroscience Branch
NIDA Addiction Research Center
P.O. Box 5180
Baltimore, MD 21224

Mechanisms of Phencyclidine (PCP)-n-Methyl-d-Aspartate (NMDA) Receptor Interactionr Implications for Drug Abuse Research

Stephen Ft. Zukin and Daniel C. Javitt

INTRODUCTION

Phencyclidine (1,1-phenylcyclohexylpiperidine; PCP; "angel dust"), originally developed as a general anesthetic in the late 1950's, was found to produce loss of consciousness and-analgesia sufficient for the performance of surgical procedures while not causing significant respiratory or cardiovascular depression (Greifenstein *et al.* 1958; Meyer *et al.,* 1959; Johnstone *et al.,* 1958). However, a significant proportion of patients subjected to PCP anesthesia developed psychotic episodes typically lasting 12 to 72 hours but occasionally as long as 7 to 10 days. These episodes were characterized by excitation, unmanagability, paranoia, concreteness of thought (Greifenstein *et al.,* 1958; Meyer *et al.,* 1959) and "maniacal episodes" (Johnstone *et al.,* 1958). Subanesthetic doses (0.1 mg/kg i.v.) doses were subsequently found to induce psychotic episodes in normal volunteers and to rekindle presenting symptomatology in recompensated schizophrenic subjects (Davies and Beech 1960, Luby *et al.,* 1959, 1962). In addition, subanesthetic doses of PCP, but not LSD, amobarbital or amphetamine, could induce abnormalities in tests of abstract reasoning, cognitive processing, attention, motor function and proprioception in normal volunteers which closely resembled those seen in patients with chronic schizophrenia (Rosenbaum *et al.,* 1959; Cohen *et al.,* 1962). Despite evoking behavioral effects apparently so dysphoric, PCP emerged as a major drug of abuse during the 1970s (Petersen and Stillman 1978) and the rate of PCP abuse increased again during the 1980s (Crider, 1985). PCP possesses powerful reinforcing and abuse-promoting effects (Balster, 1986). The key to developing interventions targeting the clinical and public-health problems posed by PCP-like drugs will be. found in elucidating the mechanisms underlying their psychotomimetic and abuse-promoting properties.

PCP binds with high affinity to a specific brain PCP receptor (Zukin and Zukin 1979; Vincent *et al.,* 19779; Reynolds *et al.* 1987). Several lines of evidence indicate that the PCP receptor is a site within the ion channel gated by the NMDA-type excitatory amino acid receptor. First, PCP and NMDA receptors are co-localized in the central nervous system (Maragos *et al.,* 1988). Second, PCP receptor ligands have been shown to inhibit NMDA receptor-mediated conductances noncompetitively (Anis *et al.,* 1983) in a voltage- and use-dependent fashion (Honey *et al.,* 1985; Huettner and Bean 1988). Finally, binding of PCP receptor ligands is enhanced by NMDA receptor agonists, such as L-glutamate or NMDA, and is diminished by competitive NMDA receptor antagonists such as D(-)-2-amino-5-phosphonovaleric acid

(D(-)AP5) (Javitt *et al.,* 1987; Fagg, 1987, Loo *et al.,* 1987). Such data suggest a model in which NMDA and PCP receptors-represent distinct sites associated with a supramolecular NMDA receptor complex. The identification of noncompetitive inhibition of NMDA receptor function as the mechanism underlying the psychotomimetic effects of PCP suggests that elucidation of the functioning of the NMDA receptor complex may reveal mechanisms relevant to the pathogenesis and treatment of schizophrenia.

MECHANISMS OF PCP - NMDA RECEPTOR INTERACTION

In order to elucidate mechanisms underlying NMDA receptor activation, binding of the selective PCP receptor ligand [^{3}H]MK-801 was determined in the presence and absence of L-glutamate and either glycine or D-serine, agents which had previously been shown to stimulate binding to PCP receptors (Reynolds *et al.,* 1987; Javitt and Zukin 1989a). L-glutamate has been shown to mediate its actions as an agonist at the NMDA recognition site while both glycine and D-serine mediate their actions at a non-strychnine sensitive glycine recognition site associated with the NMDA receptor complex For these studies, rat forebrain homogenates were subjected to extensive. washing, freezing and thawing in order to reduce the high endogenous concentrations of L-glutamate and glycine present in crude brain homogenate. Specific binding of [^{3}H]MK-801 was determined at 12 - 16 time points between 5 min and 24 hr using a filtration radioreceptor assay in the presence of a 5 mM Tris buffer system adjusted to pH 7.4 and a low (30 μM) concentration of Mg^{2+} (Javitt and Zukin 1989b).

These studies resulted in three novel findings. First, analysis of association curves using a computer-assisted, non-linear curve fitting technique revealed the presence of two distinct components of [^{3}H]MK-801 binding: a fast component with a $t_{½}$ of approximately 5 min and a slow component with a $t_{½}$ of approximately 3 hrs (figure 1). This suggests that PCP-like agents do not gain access to their receptor only via open channels, since channel-blocking drugs which interact exclusively with open channels should manifest single exponential association and dissociatiqn (Starmer and Grant 1985). A model of PCP-NMDA receptor interaction consistent with bi-exponential association of [^{3}H]MK-801 would be one in which PCP-like agents can gain access to their recognition site via two distinct paths, each corresponding to one of the observed kinetic components of binding. It has been suggested that channel blocking drugs having pK_a values near physiological pH may associate with their biding sites via both fast, hydrophilic and slow, hydrophobic paths *(Ibid.).* At pH 7.4 MK-801 (pKa=8.2 (Huettner and Bean 1988)) would exist in both deprotonated and protonated forms. The former would be capable of association via both hydrophilic and hydrophobic paths while the latter would be capable of association only via a' hydrophilic path. When channels were maximally activated in the presence of combined L-glutamate and glycine, we found that >90% of [^{3}H]MK-801 binding displayed fast kinetics of association, suggesting that the fast path represents binding of [^{3}H]MK-801 to its receptor following diffusion to the binding site via a path corresponding to the open NMDA receptor channel. In the absence of added L-glutamate or in the presence of D(-)AP5, >99% of [^{3}H]MK-801 binding displayed slow kinetics of association, suggesting that the slow path represents binding of [^{3}H]MK-801 following diffusion to the binding site via a path associated with closed NMDA receptors channels. The latter path could involve diffusion of deprotonated [^{3}H]MK-801 through the lipid bilayer, through hydrophobic domains of the receptor complex or through the (closed) NMDA receptor gating mechanism. Bi-exponential kinetics of association of [^{3}H]MK-801 in the presence of L-glutamate alone indicate that association via fast and slow paths can occur simultaneously, supporting the concept that different underlying processes must be involved. The ability of PCP-like agents to reach their binding site within closed NMDA channels over the course of

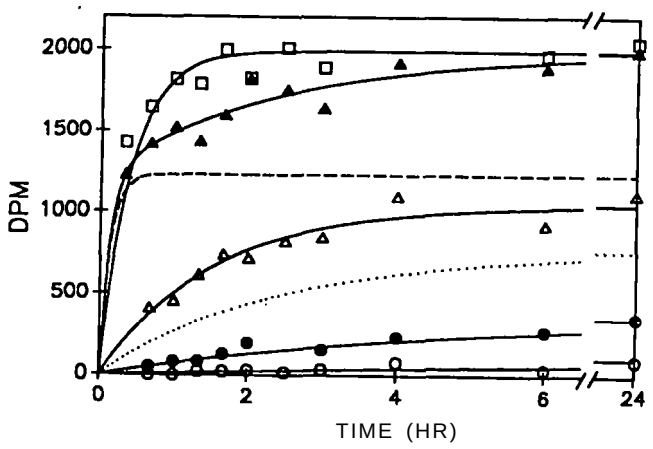

FIGURE 1: *Association curves of 1 nM [^{3}H]MK-801 under control conditions (filled circles) or in the presence of 10 µM concentrations of D(-)AP5 (open circles), glycine (open triangles), L-glutamate (filled triangles), or L-glutamate plus glycine (open squares). Under control conditions or in the presence of D(-)AP5, binding was fit best by single exponentials with apparent $t_{½}$ values of 3.2 and 5.8 hours, respectively. In the presence of combined L-glutamate and glycine, binding was best fit by a single exponential with an apparent $t_{½}$ of 12 minutes. In the presence of L-glutamate alone, binding was best fit by dual exponentials with apparent t, values of 5.5 minutes and 1.6 hours for the fast (dashed line) and slow (dotted line) components, respectively (Reprinted from Javitt and Zukin 1989b; Copyright 1989, American Society for Pharmacology and Experimental Therapeutics).*

hours may be relevant to course of PCP intoxication since it suggests that PCP can reach its site of action even in the absence of NMDA receptor activation.

A second finding of these studies (Javitt and Zukin 1989b) was that L-glutamate significantly increased total steady-state [^{3}H]MK-801 binding' while D(-)AP5 significantly decreased total steady-state [^{3}H]MK-801 binding (figure 2), presumably by displacing endogenous agonists from the NMDA receptor. This finding differs from those of previous studies which had reported no change in equilibrium binding following the addition of glutamate and glycine (Kloog *et al.,* 1988; Bonhaus and McNamara 1988). A third finding of our studies (Javitt and Zukin 1989b) was that the Hill coefficient for stimulation of [^{3}H]MK-801 binding by L-glutamate was significantly greater than unity (figure 3). While glycine shifted the dose-response curve to the left, it did not alter the Hill coefficient significantly. This finding suggests that more than one molecule of agonist is required to induce NMDA channel activation.

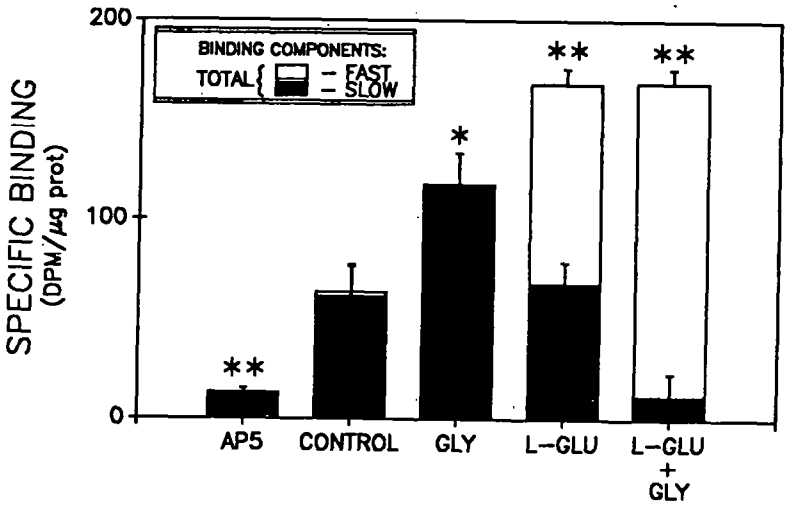

FIGURE 2: *Specific binding (mean ± s.e.m) of 1 nM [^{3}H]MK-801 to fast (open bar) and slow (filled bar) components under control conditions or in the presence of 10 µM concentrations of D(-)AP5 (AP5), glycine (GLY), L-glutamate (L-GLU) or L-glutamate plus glycine. Total bar height represents total steady-state binding of [^{3}H]MK-801 under conditions specified. Significant between-groups variation was found for fast ($p<.001$), slow ($p<.01$) and total ($p<.001$) steady-state binding. Values represent mean ± s.e.m of 4 - 6 expeiments. *$p<.05$ vs. control **$p<.01$ vs. control (Reprinted from Javitt and Zukin 1989b; copyright 1989, American Society for Pharmacology and Experimental Therapeutics).*

A model of NMDA receptor functioning which was could account for these findings is one involving the existence of two independent sets of agonist recognition sites within each functional NMDA receptor complex Occupation of both sets by agonist would be required for channel activation and fast [^{3}H]MK-801 binding. Partial activation would not permit channel opening but would permit slow diffusion of [^{3}H]MK-801 to its binding site via a hydrophobic path. In the total absence of agonist, however, the channel would remain closed and in a conformation to which PCP receptor ligands could not bind. This model is similar to models which have been proposed to account for the functioning of nicotinic acetylcholine (Hess *et al.,* 1983) and GABA, (Aoshima *et al.,* 1987) receptors. If future studies confirm a functional similarity between NMDA and nicotinic receptors, it may indicate that NMDA receptors are structurally homologous to receptors of the Class I superfamily of ligand-gated channels, which includes nicotinic, GABAA, and strychnine-sensitive glycine receptors as well (Barnard *et al.,* 1987).

ROLE OF NMDA RECEPTORS IN PSYCHOTOMIMETIC EFFECTS OF PCP

The ability of PCP-like agents to bind with high potency to a site within the NMDA channel suggests that PCP-induced NMDA channel blockade may be relevant to the clinical effects of PCP. The degree. to which PCP receptors mediate the psychotomimetic effects of PCP has been a subject of controversy in the literature.

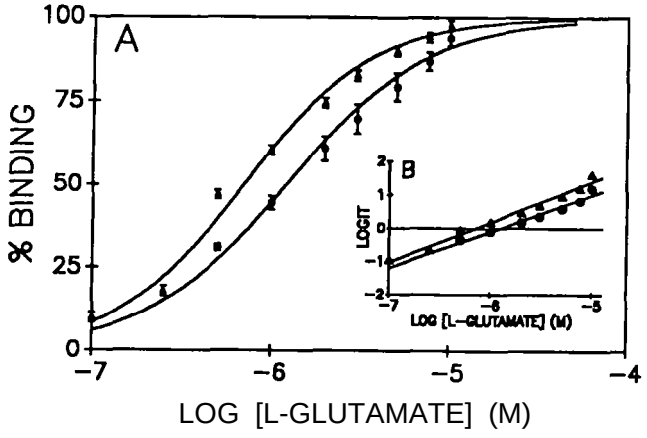

FIGURE 3: *Stimulation of specific 1 nM [^{3}H]MK-801 binding by L-glutamate in the absence (filled circles) or presence (filled triangles) of 10 µM glycine following 24 hour incubations. D(-)AP5 (50 µM) was added under all conditions to decrease basal binding. Inset, Hill plots of specific 1 nM[^{3}H]MK-801 binding Correlation coeficients (r) were >.95 for both plots. In both the absence (filled circles) and presence (filled triangles) of 10 µM glycine Hill coefficients were significantly greater than unity. (Reprinted from Javitt and Zukin 1989b; copyright 1989, American Society for Pharmacology and Experimental Therapeutics).*

Both haloperidol-sensitive σ (Su *et al.,* 1988) and monoamine reuptake (Smith *et al.,* 1977; Garey and Heath 1976) sites have been proposed as potential alternative sites for mediation of the psychotomimetic effects of PCP. Several lines of evidence, however, support a unique role of the PCP receptor in this regard. First, PCP receptors have been shown to mediate the discriminative stimulus effects of PCP in rodents (Browne, 1986). The rank order of potency with which a large number of drugs from distinct chemical classes can induce the PCP response in animals trained to discriminate PCP from saline corresponds to their rank order of binding to PCP receptors and inducing NMDA receptor blockade *(Ibid.).* By contrast, agents which selectively bind to the σ and/or dopamine reuptake sites neither induce PCP-like discriminative stimulus effects nor antagonize the discriminative stimulus effects induced by PCP receptor ligands (Ibid.). These findings suggest that PCP receptors mediate the interoceptive cues induced by PCP and PCP-like agents. Second, psychotomimetic effects similar to those induced by PCP can be induced by ketamine, a related arylcyclohexylamine derivative (Siegel, 1978). The psychotomimetic effects of ketamine are induced by doses approximately 10-fold greater than PCP *(Ibid.),* which is consistent with its ten-fold lower potency of binding to PCP receptors (Zukin and Zukin 1979, Vincent *et al.,* 1979). By contrast, ketamine is essentially inactive at both DA reuptake (Vignon *et al.,* 1988) and σ sites. Finally, PCP-induced psychosis has been found to be associated with serum concentrations of PCP as low as 20 nM, while serum concentrations greater than 400 nM are associated with gross impairments in consciousness (Walberg *et al.,* 1986). PCP receptors have been shown

to bind PCP with an affinity of approximately 30 - 50 nM (Reynolds *et al.,* 1987; Sircar *et al.,* 1987; Wong *et al.,* 1988), suggesting a highly significant degree of receptor occupancy by levels of PCP present during low-dose PCP psychosis. By contrast, σ binding sites and DA reuptake sites have been shown to bind PCP with affinities of approximately 600 (Wong *et al.,* 1988) and 700 (Vignon *et al.,* 1988) nM, respectively. The affinity of PCP for these sites is thus significantly lower than its affinity for PCP receptors. Furthermore, the affinity of PCP for these sites suggests that they would be affected only to a limited extent by concentrations of PCP that have been demonstrated to cause robust psychotomimetic effects.

In summary, the unique behavioral effects of PCP and related drugs, which are accompanied by a high degree of abuse potential, appear to be mediated at the PCP receptor which is located within the NMDA-receptor gated ion channel. Our experimental data from experiments measuring the effects of NMDA receptor activation on binding of [^{3}H]MK-801 to PCP receptors support a model of NMDA receptor functioning in which two molecules of agonist are required for NMDA receptor activation. This model is similar to models which have been proposed for the nicotinic acetylcholine receptor, suggesting the possibility of functional and structural homology between NMDA receptors and members of the Class I superfamily of ligand-gated channels. The psychotomimetic effects of PCP are observed at serum concentrations similar to the concentration at which PCP binds to the NMDA-associated PCP receptor. It is hoped that future studies may clarify mechanisms whereby agents influencing NMDA receptor functioning may influence behaviors or lead to therapeutic approaches related to drug abuse.

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ACKNOWLEDGEMENTS

This work was supported in part by USPHS grants DA-03383 to SRZ and MH-00631 to DCJ, grants from the Ritter Foundation and the David Berg Family Fund for Research in Manic Depressive Illness to SRZ, and by the generous support of the Department of Psychiatry of the Albert Einstein College of Medicine, Herman M. van Praag, M.D., Ph.D., Chairman.

AUTHORS

Stephen R. Zukin, M.D.
Daniel C. Javitt, M.D.
Departments of Psychiatry and Neuroscience
Albert Einstein College of Medicine and Bronx Psychiatric Center
1300 Morris Park Avenue, F111
Bronx, NY 10461

Characterization of the Actions of Phencyclidine on Midbrain Dopamine Neurons

Edward D. French, Stefanie Levenson and Angelo Ceci

INTRODUCTION

The dopaminergic A_{10}-mesolimbic-mesocortical systems originating within the ventral tegmental area (VTA) are increasingly implicated as playing a seminal role in reward mechanisms associated with drugs of abuse (Wise and Bozarth, 1987). Thus, the stimulation of these neurons by PCP and the a-benzomorphan compounds seemed a likely mechanism by which these drugs exert their reinforcing as well as psychotomimetic effects. Since there is compelling evidence for the existence of two distinct receptor populations for PCP and a-ligands, with both PCP and σ drugs having affinity for both sites, it is important to determine which receptor population is linked to the activation of these neurons, and whether these effects might share a common mechanism of action, such as blockade of central NMOA receptors. In this report we will provide data that compares and contrasts the potency of various PCP- and σ-ligands for effecting changes in A_{10} firing, and whether these effects, which might reside with PCP's noncompetitive blockade of NMDA receptors, are shared by those compounds which competitively antagonize central NMOA receptor activation. We will also present evidence that PCP's unique bimodal effect (excitation/inhibition) on A_{10} activity is mediated via independent transmitter systems.

METHODS

Electrophysiology

All experiments were carried out on male Sprague-Dawley derived rats according to procedures reviewed and approved by the Univ. Arizona IACUC Committee according to established federal regulations for the treatment of animals. The preparation, single-unit extracellular recordings and identification of presumptive dopamine neurons in chloral hydrate anesthetized rats has been described in detail elsewhere (French, 1986, 1988). Challenge drug injections were administered i.v. according to a cumulative dosing regimen while continuously monitoring changes in A,, cell firing rate.

Behavior

The locomotor effects of PCP (5 mg/kg), d-amphetamine (AMP, 1.5 mg/kg), scopolamine (1.25 mg/kg) and the competitive NMDA antagonist, NPC 12626 (50 mg/kg) in rats with 6-hydroxydopamine lesions (8µg/2µl/side) of the nucleus accumbens were measured in photocell cages as previously described (French and, Vantini, 1984).

Lesions

Animals were prepared with unilateral excitotoxic lesions of the nucleus accumbens (kainic acid, 2µg/O.75µl), medial prefrontal cortex (N-methyl-D-aspartic acid, 25µg/0.6µl) or radiofrequency lesions of the dorsal raphe nucleus. Also, the neurotoxin parachloroamphetamine (PCA, 5 mg/kg/day for 2 days) was used to deplete central serotonin (5-HT) levels. Subsequent electrophysiological assessments were begun after a recovery period of 7-10 days in the lesioned animals and 24 h after the last injection of PCA.

RESULTS

A series of drugs covering the spectrum from high-affinity PCP-receptor ligands (MK-801) to high-affinity u-receptor ligands (ditolylguanidine (DTG)) were tested for their ability to alter the firing rate of A,, DA neurons. As can be seen in figure 1, MK-801 which has been shown to have the highest affinity and selectivity for the PCP receptor (Wong et al, 1986) was the most potent of all the compounds in activating dopamine cell firing, followed by PCP, (+)SKF 10047 ((+)SKF) and ketamine (KET). The maximal activation elicited by MK-801, PCP,(+)SKF and KET was approximately 40%. Moreover, only PCP showed an inflection in the dose-response profile with a slowing component emerging at doses >1 mg/kg.

Of the highly u-preferring compounds (+)pentazocine ((+)PTZ) produced a maximum increase of only 15%, while DTG failed to produce any activation of firing. Calculations of relative potency (Tallarida and Murray, 1987) found MK-801 to be 2, 4, 10 and 80 times more potent than PCP, (+)SKF, KET and (+)PTZ, respectively in evoking increased firing. Thus, it would appear that activation of the DA neurons comprising the mesolimbic-mesocortical system is mediated via PCP, not u-receptors. The fact that (+)PTZ and DTG, high-affinity ligands for the haloperidol-sensitive u-site (Weber et al., 1986), with little to no affinity for the PCP receptor, were virtually devoid of effects on these neurons supports this conclusion. Furthermore, the activation of A,, neurons by PCP, (+)SKF and MK-801 was not attenuated by haloperidol (Freeman &. Bunney, 1984; French, 1986, 1989) which has the highest known affinity for the σ-site.

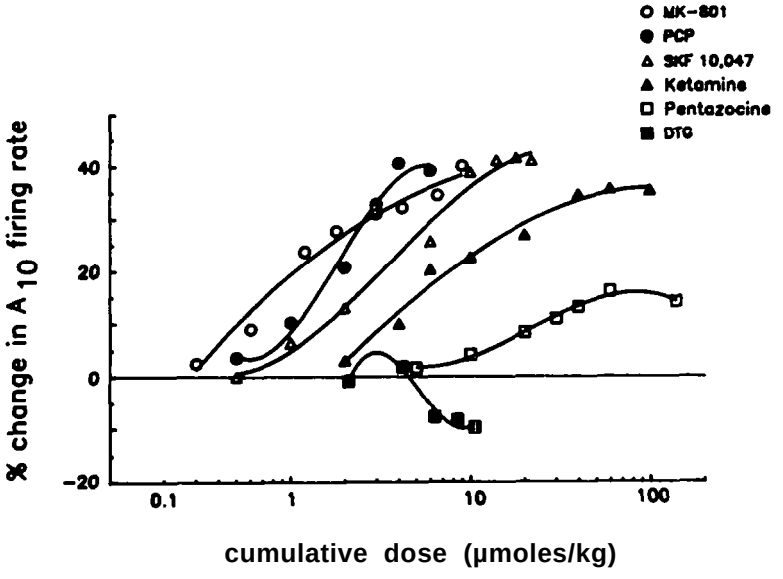

FIGURE 1. Cumulative dose-response effects of PCP and u-drugs on firing rate of A,, neurons following i.v. administration.

The potency of PCP-like drugs to alter A,, activity was found to correlate positively (r=0.97) with their affinity for the PCP receptor and consequently with their potency as NMDA antagonists (Aram et al., 1986). Whether blockade of central NMDA receptors on mesolimbic-mesocortical DA neurons is the primary mechanism by which PCP and PCP-like drugs produce their psychopharmacological effects remains an intriguing issue. Although directly acting NMDA antagonists have been shown to elicit PCP-like behaviors (Koek et al, 1986), there is some debate as to how PCP-specific these NMDA actions are (Willette and Balster, 1988). Since PCP and related drugs appear to have a common electrophysiological effect on A neurons, the response of these cells to directly acting NMDA antagonists might indicate their potential for having psychotomimetic properties. In these experiments we measured changes in A,, firing during cumulative dosing with i.v. injections of NPC 12626 (60 mg/kg) and (±)CPP (40 mg/kg), both high affinity selective NMDA blockers (Davies et al., 1986; Ferkany, personal communication). Both compounds failed to significantly change firing rate up to 45 min. from the last injection. The maximum effect elicited by NPC was +2.2% and that of (±)CPP +8.2%. Moreover, following CPP infusions subsequent challenge with incremental doses of PCP failed to increase activity more than 10%; a marked attenuation of PCP's normal 40% plus degree of activation. In the behavioral assessment we did find that NPC 12626 (50 mg/kg, i.p.) produced a significant degree of locomotor

hyperactivity accompanied by some ataxia. However, these effects, like that of scopolamine were not sensitive to 6-OHDA lesions of the accumbens; in marked contrast to the significant reductions in PCP and d-AMP-induced activity in these lesioned animals (Table 1). Thus it would appear that mesolimbic presynaptic DA mechanisms do not mediate the PCP-like behaviors produced by competitive NMDA antagonist.

TABLE 1

Photocell counts

Challenge drug	Sham	6-OHDA	P-value
Saline	244 ± 34	382 ± 70	NS
PCP (5 mg/kg	855 ± 77	502 ± 88	0.01
NPC 12626 (50 mg/kg)	677 ± 84	627 ± 107	NS
d-AMP (1.5 mg/kg)	1562 ± 127	718 ± 92	0.01
SCOP (1.25 mg/kg)	550 ± 56	723 ± 87	NS

Unlike the other drugs we have tested PCP is unique in that it produces a bimodal (excitation/ inhibition) effect on A,, cell firing. Since the mesolimbic nucleus accumbens and mesocortical prefrontal cortex contain relatively high concentrations of PCP binding sites as well as provide inhibitory and excitatory inputs to the VTA, respectively (Wolf et al., 1979; Christie et al., 1985), we examined the effects of excitotoxic lesions of these two areas on the response of A_{10} cells to PCP. As can be seen in fig. 2, the downward component normally produced by PCP at doses >1 mg/kg is eradicated in accumbens lesioned animals and replaced with a significantly elevated and sustained level of activity (88% in kainate and 55% in electrolytic lesioned animals). However, at doses <1 mg/kg neither basal firing rates nor the magnitude of excitation differed from controls. In contrast, destruction of the medial prefrontal area failed to alter the dose-response characteristics to PCP. These results, while strongly implicating accumbal feedback systems in the inhibitory component of PCP action also indicate that medial prefrontal excitatory amino acid afferents to VTA are apparently not involved in the PCP-induced excitation of A_{10} firing. Thus, it seemed reasonable to conclude that the low-dose stimulating effects of PCP were operationally determined by (an)other system(s).

Since the dorsal raphe nucleus provides substantial input to the VTA and serotonin (5-HT) has been implicated in some PCP-induced behaviors (Nabeshima et al., 1984) we next focussed on the possible involvement of this transmitter system in the PCP-induced activation of A_{10} neurons. In these experiments we found that disruption of central 5-HT function by radiofrequency lesions of the dorsal raphe (5-HT: 54% of control) or depletion of 5-HT by

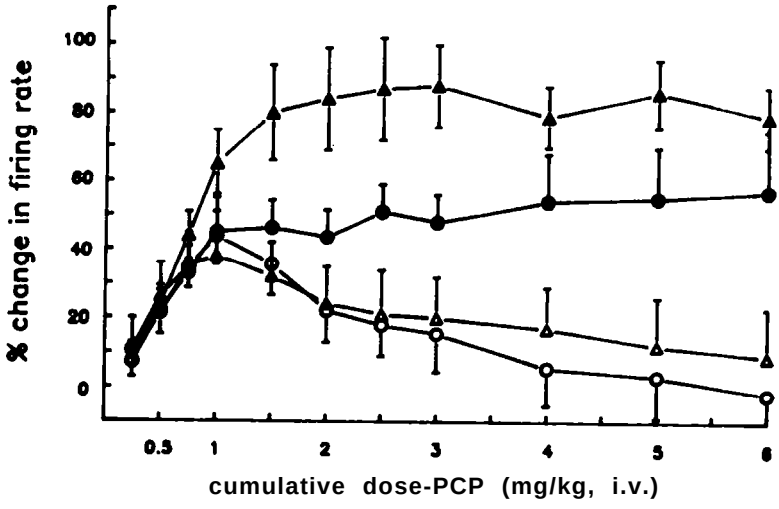

FIGURE 2. Response of A_{10} dopamine neurons to PCP in animals with kainate (▲), or radiofrequency (●) lesions of the nucleus accumbens, or N-methyl-D-aspartic acid (△) lesions of the medial prefrontal cortex. The response of the control groups were not different and were therefore combined (○). N=6-9 animals/group.

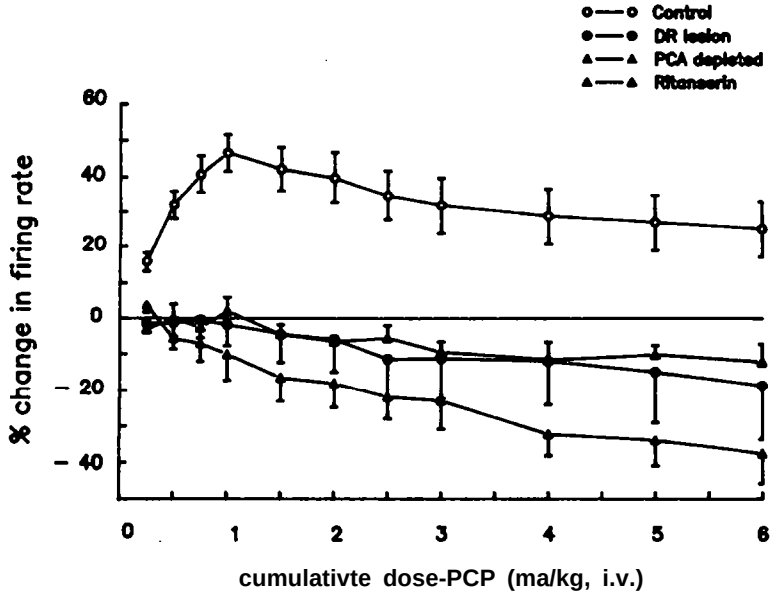

FIGURE 3. Attenuation of the response of A_{10} neurons to PCP following disruption of central 5-HT function.

parachloroamphetamine pretreatment (5 mg/kg once every 24 hr for 2 days) (11% of control) completely prevented PCP's low-dose excitatory effects (fig. 3). The fact that pretreatment with the 5-HT_2 selective antagonist ritanserin (5 mg/kg, i.p. 1-4 hr before) also blocked PCP-induced activation of A_{10} neurons strongly suggests a role for this 5-HT receptor subtype in this response. More recently, we have also observed that 8-OH-DPAT, a 5-HT_{10} agonist which is a potent inhibitor of 5-HT cell firing, also antagonized the activating effects of PCP (fig. 4). Thus, it seems reasonable to conclude that PCP's primary low-dose stimulatory effects on A_{10} DA neurons are mediated via an interaction with 5-HT afferents to the VTA. The precise mechanism by which this occurs and the potential involvement of the median raphe 5-HT system is being investigated further.

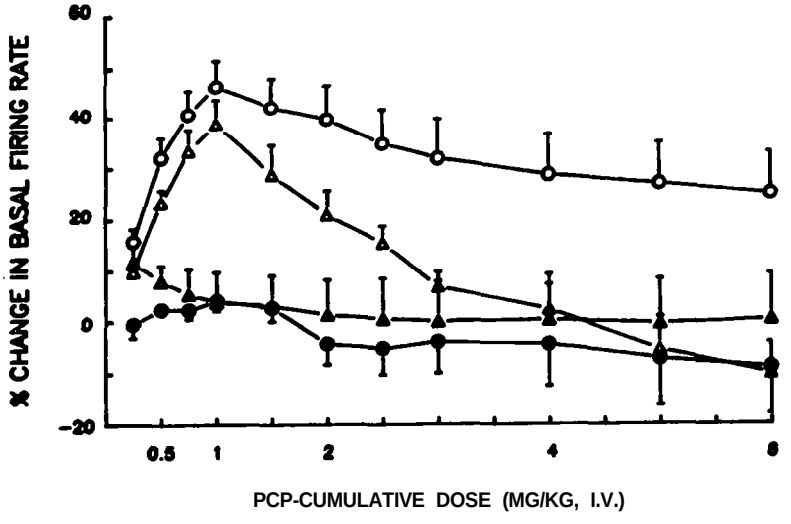

FIGURE 4. Response of VTA and substantia nigra pars compacta DA neurons to PCP following saline (○, △) or 8-OH-DPAT (30 ug/kg) (●, ▲) pretreatment, respectively.

DISCUSSION

The data presented here clearly establish a positive correlation between the ability of PCP and PCP-like drugs to activate A_{10} DA neurons and their affinity for central PCP receptors. The ineffectiveness of high affinity u-specific ligands to alter DA cell firing further argues against the participation of u-receptors in the stimulation of these A_{10} neurons by PCP or σ-psychotomimetics. Since the potency of these drugs to stimulate DA activity also correlated with their potency as non-competitive NMDA receptor antagonists (MK-801>PCP>(+)SKF 10047>KET)(Kemp et al., 1987), we sought to determine if competitive blockers of glutamate action at NMDA sites would also mimic PCP's effects. Our data show that this is not necessarily the case since rather large i.v. doses

of direct NMDA antagonists (NPC 12626 and (±)CPP) did not stimulate A_{10} activity. Although it could be argued that these phosphonic acid compounds do not readily enter into brain, this seems unlikely since a comparable dose of NPC 12626 administered i.p. elicited both locomotor hyperactivity and ataxia. Furthermore, (±)CPP pretreatment markedly diminished PCP-induced activation of A_{10} neurons, much in accord with radioligand binding studies showing that a competitive antagonist can lower binding to the PCP receptor (Javitt, et al., 1987; Kemp et al., 1987; Reynolds et al, 1987). Also, systemic doses less than those used here have been shown to be anticonvulsant, anti-ischemic and to produce some PCP-like behaviors in drug discrimination paradigms. However, antagonism of microiontophoretic NMDA-induced neuronal excitations by systemically administered antagonists will be needed to definitively settle this issue. In addition, our data showing that NPC 12626-induced hyperactivity is not sensitive to 6-OHDA lesions of the nucleus accumbens is also another indication that while competitive blockade of the NMDA receptor complex produces some PCP-like behaviors, these effects are not mediated through a common site of action: the mesolimbic dopaminergic pathway.

Our studies into the sites and mechanisms by which PCP produces its rather unique dose-dependent bimodal effect also produced some interesting findings which strongly suggest that these two phenomena are independently subserved through different CNS transmitters. The low dose excitations are sensitive to disruption of serotonergic transmission possibly originating in the dorsal raphe nucleus, while the higher dose slowing component may involve a negative feedback pathway to the VTA from the nucleus accumbens.

In conclusion, we have shown that the activation of mesolimbic-mesocortical dopamine containing systems by PCP, and the σ-benzomorphans is mediated by the PCP receptor. If the midbrain DA neurons are crucially involved in the reinforcing as well as psychotomimetic properties associated with both classes of drugs, then it would seem a logical conclusion that PCP receptors play the pivotal role in these actions.

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ACKNOWLEDGMENTS

These studies were support in part by National Institute on Drug Abuse grant DA 03876 and Neuroscience Center for Research in Schizophrenia grant MH 44211.

AUTHORS

Edward D. French, Ph.D.
Stefanie Levenson
Angelo Ceci, Ph.D.

Department of Pharmacology
University of Arizona
College of Medicine
Tucson, Arizona 85724

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Cerebral Pathways Activated by PCP-Like Compounds: Relevance to Neurotransmitters and Their Receptors

M.F. Piercey and W. E. Hoffmann

Phencyclidine (PCP, "angel dust") is a hallucinogenic drug of abuse which, when taken in large doses, produces a schizophreniform syndrome that is easily mistaken for positive symptom schizophrenia (Luisada, 1978). Consistent with the widely held theory that schizophrenia is a hyperdopaminergic state, PCP evokes release of the neurotransmitter dopamine and induces certain dopaminergic behavioral effects such as stereotypy in animals (Domino and Kamenka, 1988). Moreover, PCP intoxication can be partially treated with antipsychotic dopamine antagonists such as haloperidol (Luisada, 1978). Evidence has accumulated that PCP and related arylcyclohexylamines are channel-blocking agents at the NMDA excitatory amino acid receptor. Thus, PCP blocks the effects of NMDA and related excitatory amino acids (Anis et al., 1983) and high affinity PCP binding sites are co-distributed with those for NMDA receptors (Maragos et al., 1986). A low affinity PCP binding site has been identified as the "opioid" sigma receptor site thought to be associated with "delirious" states (Sircar et al., 1986).

We have utilized Sokoloff's (1977) 2-deoxyglucose (2-DG) autoradiographic-methods to identify which brain pathways are altered by PCP. The techniques have been previously detailed (Piercey et al., 1988a). Briefly, 25 µCi 2-DG is injected in the saphenous brain 45 minutes prior to sacrifice. Animals are restrained over this time either in a plaster cast or, more recently, an animal restrainer, to allow periodic sampling of arterial blood for inclusion of plasma radioactivity and glucose into the Sokoloff equations for estimating brain glucose metabolism from autoradiograms. While drug effects are unaltered, the increased activity allowable in the restrainer tubes has resulted in increased control metabolic rates (compare table 1 with tables 2 and 3). Following sacrifice, the brains are removed, rapidly frozen, sectioned on a cryostat, autoradiograms developed, and analyzed by area analysis on a Loats image analyzer.

Utilizing this method, PCP was found (Piercey and Ray, 1988a,b) to induce dramatic stimulation of brain forebrain pathways, particularly dopaminergic structures, Papez' (1937) limbic circuit, and some parts of the cerebral cortex (Table 1).

TABLE 1. Effects of PCP (5 mg/kg) and Haloperidol (0.1 mg/kg) on Regional Glucose Metabolism in the Rat Brain.

Structure	(µmole glucose/100 g/min) Control	PCP	PCP+Hal
Cerebral Cortex			
Olfactory Cortex	85±5	126± 6*	108±11*
Dorsal Prefrontal	72±3	85± 6*	69± 5^{+}
Frontal Cortex	73±4	77± 7	59± 4*,$^{+}$
Motor Cortex			
Dense column	75±3	94± 7*	70± 5
Light column	75±3	49± 3*	45± 3*
Sensory Cortex			
Dense column	87±5	63± 2*	56± 4*
Light column	87±5	51± 5*	45± 4*
Piriform Cortex	83±3	103± 6*	96± 4
Parietal Cortex	81±5	93± 3	80± 5
Auditory Cortex	111±4	103± 5	93± 5*
Striate Cortex 17	81±4	81± 3	71± 3
Striate Cortex 18	76±4	115± 5*	102± 8*
Papez Circuit			
Mamillary Body	85±5	222±10*	212± 6*
AV Thalamus	90±8	214± 5*	220± 5*
Anterior Cingulate	78±3	111± 3*	105± 7*
Entorhinal Cortex	50±2	61± 2*	57± 3
Hippocampus			
Dentate	47±3	79± 5*	76± 5*
Dendritic Zone	66±3	132± 7*	129± 5*
Pyramidal Layer	41±1	42± 4	41± 1
Fornix	32±4	43± 3*	43± 1*
Dopaminergic Zones			
M Prefrontal Cortex	107±5	159± 2*	138± 3*,$^{+}$
Caudate	77±2	127± 5*	117± 2*
N Accumbens	53±3	95±10*	73± 6*,$^{+}$
Olfactory Tubercle	69±3	85± 7*	75± 6^{+}
Globus Pallidus	46±2	80± 2*	65± 4*,$^{+}$
Substantia Nigra	46±2	137±12*	76± 4*,$^{+}$
VTA	53±3	52± 5	51± 2

Plaster *cast restrainers, n=4* except *for controls where n=8.*
* *significantly different from controls, (p<0.05)*
$^{+}$ *significantly different from PCP (p<0.05)*

Depressed areas include the sensory cortex, lateral habenula, and the inferior colliculus. A rather remarkable effect is the induction of vertical light/dark metabolic columns in the motor cortex, possibly due to repeated use of specific motor pathways associated with stereotypic behaviors (Piercey and Ray, 1988a,b).

The stimulant effects of PCP in the dopaminergic zones are partially antagonized by haloperidol (table 1), reminiscent of

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the weak effects this agent has in treating overdoses in man. Non-dopaminergic areas are unaffected by haloperidol (Piercey and Ray, 1988b). Amphetamine, a schizophreniform agent which acts primarily through stimulation of dopamine release, mimics PCP in stimulation of dopaminergic areas but does not at all stimulate Papez circuit (table 2). Amphetamine does not stimulate the cerebral cortex. The inability of haloperidol to dramatically inhibit PCP's affects or amphetamine to mimic most of them indicates that much of PCP's effects do not involve dopamine. However, stimulation of dopaminergic regions could be responsible for some of the psychotomimetic as well as reinforcing properties of PCP.

TABLE 2. Effects of Amphetamine (10 mg/kg) on Regional Glucose Metabolism in the Rat Brain.

Structure	(µmole glucose/lOO g/min) Control	Amphetamine
Cerebral Cortex		
Olfactory Cortex	154±14	154±12
Dorsal Prefrontal	148±10	158±12
Motor Cortex	158± 5	176±17
Sensory Cortex	166± 6	211±20
Piriform Cortex	165± 8	150± 8
Parietal Cortex	158± 4	167± 9
Auditory Cortex	181± 9	190±11
Striate Cortex 17	182± 6	147±11*
Striate Cortex 18	174± 7	147±12
Retrosplenial Cortex	141± 6	114±10
Papez Circuit		
Mammillary Body	158±11	154±14
AV Thalamus	151± 4	145±14
Anterior Cingulate	156± 5	146±13
Entorhinal Cortex	102± 5	100± 9
Hippocampus		
Dentate	84± 5	64± 4*
Dendritic Zone	136± 9	119± 8
Pyramidal Layer	100± 7	91± 4
Fornix	69± 3	85± 5*
Dopaminergic Zones		
M Prefrontal Cortex	141± 8	146±14
Caudate	164± 5	226±17*
N Accumbens	82± 8	116± 7*
Olfactory Tubercle	139± 6	172± 9*
Globus Pallidus	92± 5	122± 8*
Substantia Nigra	74± 7	147±15*
VTA	101± 7	135± 9*

Animal tube restrainers, n=4
** significantly different from controls, (p<0.05)*

TABLE 3. Effects of CPP (100 mg/kg) and MK-801 (1 mg/kg) on Regional Glucose Metabolism in the Rat Brain.

Structure	(µmole glucose/100 g/min) Control	CPP	MK-801
Cerebral Cortex			
Olfactory Cortex	139±11	164±10	237± 7*
Dorsal Prefrontal	143± 7	124± 5	153± 8
Motor Cortex	143±14	129± 8	164±13
Sensory Cortex	162±11	142± 7	138±11
Piriform Cortex	158± 6	174± 7	224±12*
Parietal Cortex	162± 9	131± 8*	181±11
Auditory Cortex	161±15	153±12	160±11
Striate Cortex 17	151±14	138± 5	176± 5
Striate Cortex 18	147±11	139± 6	212± 5*
Retrosplenial Cortex	131±12	96± 3*	292±13*
Papez Circuit			
Mammillary Body	152± 6	151± 8	321± 9*
AV Thalamus	145± 3	131± 9	292±15*
Anterior Cingulate	157±13	115± 6*	228±14*
Entorhinal Cortex	93± 5	95± 4	182± 1*
Hippocampus			
Dentate	83± 3	76± 5	158±10*
Dendritic Zone	125± 2	121± 6	212±13*
Pyramidal Layer	91± 3	79± 6	86± 5
Fornix	40± 4	59± 4*	85±10*
Dopaminergic Zones			
M Prefrontal Cortex	133± 9	119± 9	200± 6*
Caudate	150± 8	156±10	231± 9*
N Accumbens	95± 7	84± 5	138± 7*
Olfactory Tubercle	139± 6	176± 9	217± 7*
Globus Pallidus	78± 6	84± 5	140± 6*
Substantia Nigra	70± 7	77± 5	105± 8*
VTA	91± 6	97± 8	125± 9*

Animal tube restrainers, n=4-7
l *significantly different from controls, ($p<0.05$)*

The severe "rage" response, which includes delusions of "super-human" strength, are a component of PCP's behavioral syndrome (Luisada, 1978) but not that for amphetamine. It is tempting to associate this intense PCP effect with this circuit that Papez suggested integrated emotional sensations and expressions.

There is a strong suggestion that PCP's effects are associated with NMDA receptors. Regions excited by PCP are endowed with high concentrations of high affinity PCP receptors. Areas containing high concentrations of sigma receptors are, in contrast, not excited by PCP (Piercey and Ray, 1988a,b). Since the high affinity PCP receptors are intimately related to NMDA

receptors (Maragos et al., 1986), PCP's effects, including those of dopaminergic areas, may be expressed through those receptors. This suggestion is further supported by the fact that PCP's effects are imitated by MK-801 (table 3), another NMDA receptor channel-blocking agent which is weaker than PCP in stimulating dopamine release (Domino and Kamenka, 1988).

It is not clear how PCP's dramatic stimulant effects could be evoked by antagonism of a strong excitatory amino acid. This question is heightened by the fact that CPP, an NMDA receptor antagonist, does not mimic the effects of PCP and MK-801 in effects on brain energy metabolism (table 3); indeed, CPP depressed metabolism in the cingulate gyrus. If PCP's stimulant effects are associated with NMDA receptors but not their blockade, then one is led to suggest that PCP somehow stimulates these receptors, perhaps by inducing them to open for short periods of time. However, electrophysiological experiments do not support this speculation (Anis et al., 1983).

Positron-emission-tomography (PET), a non-invasive technique, allows 2-DG analysis in living, human patients. PET scans of schizophrenic patients consistently reveal a metabolic gradient ("hypofrontanality") increasing from frontal to occipital cortex (Buchsbaum, 1985). Some hypofrontanality is observed also in PCP-intoxicated patients (Dr. Joseph Wu, personal communication). PCP-treated animals also show some hypofrontanality (compare frontal to sensorimotor to parietal and then striate cortex, table 1). However, unlike PCP-treated animals, predominant metabolic effects in schizophrenic and PCP-intoxicated patients are depressions; only the occipital cortex region is stimulated. The reasons for these differences between animal and human studies is not clear, but could be due to the need to maintain patients in a relatively quiet state in order to obtain PET-scans.

The pathways and neurotransmitters associated with behavioral effects of PCP are beginning to be revealed. Resolution of the perplexing contradictions could lead to new insights into psychiatric states, including drug abuse.

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AUTHORS

Montford F. Piercey, Ph.D. and William E. Hoffmann, M.S., CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

Behavioral Pharmacology of PCP, NMDA and Sigma Receptors

Robert L. Balster

The effects of PCP in animals has been proposed as a model of schizophrenia. There are a number of rationales for this proposal, including: a) the similarities between the PCP psychosis which occurs in some PCP abusers and schizophrenic disease; b) the ability of PCP to exacerbate schizophrenia; and c) certain commonalities in the altered perceptions and thought processes of persons under the influence of PCP and schizophrenic patients. The neuropharmacological rationale for the model includes the observation that PCP has a number of dqpaminergic actions, and alterations in dopamine function have been hypothesized to occur in schizophrenia. Therefore, studies of the neural basis for PCP intoxication in animals may provide clues to the underlying pathology present in psychosis.

PCP is now thought to produce many of its behavioral and pharmacological effects through actions at a specific receptor in the central nervous system. This PCP receptor has been the subject of intensive research. One of the early findings was that this receptor may also mediate the effects of certain opioids which also produced psychotomimetic effects. These opioids were hypothesized by W. R. Martin and his colleagues to act upon an opiate receptor subtype referred to as the sigma receptor, so-named because the prototypic agonist was SKF-10, 047, or N-allylnormetazocine (NANM). Indeed, early research suggested that the sigma receptor was not an opioid receptor at all, but rather was identical to the PCP receptor. This was supported by biochemical data showing common binding sites for PGP-like drugs and sigma agonists and by behavioral data showing considerable similarities in their effects.

Results of recent research have complicated this situation considerably. Binding studies with the radiolabeled sigma-agonist (+)-N-allylnormetazocine, and with other ligands, demonstrated the existence of a high-affinity binding site which differed in many ways from the PCP receptor. Although PCP also had some affinity for this site, the pattern of displacement by other drugs, as well as other data, provide clear evidence that this site is distinct from the PCP site.

Interestingly, haloperidol and some other antipsychatic drugs have a high affinity for this site but not for the PCP receptor. The fact that PCP and some psychotomimetic opioids and antipsychotic drugs bind to this site has led to speculation that this site may also play a role in PCP intoxication and/or schizophrenic disease. Although this may prove to be the case, considerable confusion presently exists in the research literature because this non-PCP site is now often referred to as the sigma receptor (Quirion et al., 1981), even though it seems increasingly clear that this is not the sigma receptor hypothesized by Martin, and is certainly not the PCP/sigma receptor which had been well characterized. Nor is it clear that the site mediates pharmacological effects of drugs which bind there, including those of sigma-agonist opioids.

Drug discrimination procedures in animals are often used as a model for subjective effects of drugs. This presentation reviewed data from my laboratory which addresses the question of which of these sites is likely to be involved in the production of the discriminative stimulus effects of PCP and (+)-N-allylnormetazocine in animals. Drugs were studied which have selective affinities for either the PCP receptor (e. g. ketamine and MK-801) or for the high-affinity, haloperidol-sensitive (+)-NANM binding site (e. g. DIG, (+)-3-PPP, (+)-ketocyclazocine and (-)-butaclamol). In rats trained to discriminate PCP from non-drug, the ability of drugs to substitute for the PCP stimulus was perfectly predicted by their ability to bind to the PCP receptor. MK-801, for example, was found to substitute completely for PCP with a potency predicted by its high affinity for the receptor (Willetts and Balster, 1988a). Although (+)-NANM also has PCP-like discriminative stimulus effects (Brady et al., 1982), it is less potent than PCP, consistent with its relative potency to PCP at the PCP receptor. Since (+)-NANM has a greater affinity than PCP for the high-affinity site, this is further evidence that this site is not responsible for the discriminative stimulus effects of PCP or (+)-NANM in this procedure. Ihis, and other evidence, provides strong support for the conclusion that the PCP receptor, and not the high-affinity (+)-NANM binding site, is responsible for discriminative stimulus effects of PCP (Balster and Willetts, 1988). The answer to the question of whether or not the "sigma receptor" plays a role in the effects of PCP and (+)-NANM depends upon which sigma receptor is being referred to. The confusing nomenclature is unfortunate.

Rats were also trained to discriminate (+)-NANM from non-drug. Haloperidol, DTG, (+)-3-PPP, (+)-ketocyclazocine and (-)-butaclamol all failed to produce (+)-NANM-like effects; nor were they able to function as antagonists of (+)-NANM (Balster, 1989). On the other hand, PCP, MK-801, ketamine, (-)-2-MDP, etoxadrol and dextrorphan all substituted fully for the (+)-NANM stimulus with a potency predicted by their relative

affinity for the PCP receptor and unrelated to their actions at the high-affinity site (most are inactive at this site).

It seems clear from the results of our studies that the high-affinity, haloperidol-sensitive (+)-NANM binding site, referred to by some as the sigma receptor, is responsible for the discriminative stimulus effects of neither FCP nor the sigma-agonist (+)-NANM. If one assumes that these effects are related to the ability of these drugs to produce a schizophrenia-like intoxication in humans, then the PCP receptor remains the cellular target of interest as possibly playing a role in psychosis and as a possible site of action for novel antipsychotic drugs. On the other hand, the high affinity of some antipsychotic drugs for the high-affinity (+)-NANM site continues to serve as a rationale for continued investigations of its possible role in psychosis.

These results bear upon recent interest in potential therapeutic uses of PCP-like drugs such as MK-801 as neuroprotectants. Certain types of neural injury, such as that caused by the ischaemia resulting from stroke, may have a basis in excitotoxicity. Glutamate is an excitatory amino acid neurotransmitter hypothesized to act upon at least three subtypes of receptors. Excessive activation of the N-methyl-D-aspartate (NMDA) receptor subtype as a result of brain insult is hypothesized to produce a calcium-dependent neural degeneration. Under many circumstances, these effects can be blocked by PCP-like drugs. Indeed, PCP has been found to be a noncompetitive antagonist of NMDA-receptor activation in many biochemical and electrophysiological models (lodge et al., 1987). Unfortunately, NMDA antagonism by PCP-receptor acting drugs is also likely to result in PCP intoxication, a side-effect which would limit their clinical utility.

NMDA antagonism can also result from drugs which act directly at the glutamate binding site to produce competitive inhibition of agonist binding. It is unknown whether NMDA antagonists acting in this way will also produce PCP-like intoxication. We have obtained data in animals showing potentially important differences in the discriminative stimulus effects of PCP-like drugs and competitive NMDA antagonists such as CPP and NPC 12626. In rats trained in a PCP discrimination, CPP (Willetts and Balster, 1988b) and NPC 12626 (Ferkany et al., 1989) fail to substitute fully for the PCP stimulus. In addition, PCP fails to substitute fully in rats trained to discriminate NPC 12626 from non-drug (Willetts et al., 1989). Finally, the competitive NMDA antagonists CPP and NPC 12626 are considerably more effective than PCP-like drugs in antagonizing NMDA discrimination (Willetts and Balster, 1989). Thus, there is a good possibility that drugs can be developed with a separation between NMDA antagonist effects and PCP-like side effects. On the other hand, these results leave unresolved the role that NMDA antagonism by PCP may play in the production of PCP intoxication, and whether interactions between PCP and NMDA

receptors would be a fruitful target for research on the neural basis of schizophrenia.

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ACKNOWLEDGEMENTS

Research supported by National Institute on Drug Abuse Research Grants DA-01442 and DA-00490. Drs. Kathleen Brady, Everette May, and Joyce Willetts made significant scientific contributions to this project.

AFFILIATION

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

Clinical Implications of PCP, NMDA and Opiate Receptors

Jefferey Wilkins

INTRODUCTION

Phencyclidine (PCP) is best known as a substance of abuse with the potential to cause psychosis and violence. Less wellknown is the recent discovery that PCP may provide the molecular basis for compounds that will treat various psychiatric and neurologic disorders. PCP binds to select central nervous system (CNS) receptors with high affinity and specificity (Zukin and Zukin 1979, Vincent et al., 1979). PCP receptors are co-distributed throughout the CNS with N-methyl-d-aspartate (NMDA) receptors (one of three excitatory ammo acid receptors [Honore 1989]) and is displaced competitively by endogenous peptides (see Zukin, this monograph). This suggests the existence of endogenous PCP ligand(s). Lodge and colleagues (1982) found that PCP noncompetitively inhibits the NMDA receptor (the NMDA receptor system is postulated to mediate cellular 'damage from stroke and epilepsy), while animal studies have shown PCP to protect against CNS damage from experimentally-induced ischemia and hypoglycemia, and block experimentally-induced seizures (see review by Honore 1989). PCP is also a psychotomimetic agent, and along with PCP agonists, causes neuronal cell death (Olney et al., 1989). PCP, PCP agonists, and presumably the endogenous PCP ligand, have the potential to both cause and prerent CNS damage. Despite these varied and complex CNS actions of PCP. evidence suggests that PCP agonists and antaeonists may be useful in the diagnosis and treatment of psychiatric disorders including schizophrenia, and as tools for defining the biochemistry of complex behaviors, including violence. This manuscript attends to the psychiatric implications of PCP's receptor actions, discussed in terms of testable hypotheses and the degree to which each hypothesis has been evaluated.

We focus on five hypotheses derived from findings and postulates of other investigators and incorporated with results from our studies. These include:

1) Administration of PCP agonists, PCP antagonists, and amphetamine, combined with biological measures, will separate schizophrenics into two groups. This classification scheme will provide clinicians with a method for selecting optimal pharmacologic treatment approaches to schizophrenia, including choosing between traditional neuroleptics versus atypical antipsychotic medications.

2) primate behavioral responses to PCP predict PCP-induced behavior in humans. That PCP induces aggression in primates but does so by first blocking social affiliative behaviors has implications for understanding violence in humans, including its biological basis. Behaviors altered by PCP in the human will be found to be a function of PCP dose, predisposition to psychosis, and social status.
3) PCP-induced protracted psychosis and post-hallucinogen perceptual disorder (DSMIIIR) are sequelae of recirculating PCP in individuals prone to psychotic responses to PCP. Treatment would include acidification of body fluids to wash out PCP from the body, thereby preventing CNS uptake of PCP through the blood-brain-barrier.
4) PCP agonists will help manage evolving stroke and early hypoglycemic shock. PCP agonists will be used as supplements in treating epilepsy that is unresponsive to conventional treatment. PCP agonists or antagonists will prove useful in treating dementia depending on the endogenous PCP ligand level.
5) neuropsychological deficits observed in PCP users are the result of PCP-mediated neurotoxic effects (Olney et al., 1989). Independent studies of PCP abusers and patients receiving ketamine will provide information on the risks of administering PCP agonists to humans.

PCP and Schizophrenia

We hypothesize that schizophrenics are divisible into two groups analagous to Crow's hypothesis of schizophrenia (Crow 1980): those with a supersensitive response to normal amounts of endogenous PCP ligand, and those with dysfunction of the feedback loop regulating PCP ligand activity, resulting in excess PCP ligand levels (see figure). The first group's supersensitive response to the PCP ligand results from excessive A10 dopaminergic activity that sensitizes the PCP receptor. The second group's elevated PCP ligand levels result in CNS toxicity as described by Olney et al. (1989) The dysfunctional feedback mechanism is analogous to hypothalamic-pituitary-adrenal (HPA) axis dysfunction in endogenous depression. **A** small dose of PCP agonist worsens positive symptoms of schizophrenics analagous to Crow's Type I, while the same dose does not affect Type II schizophrenics. **A** large dose of PCP agonist further worsens Type I positive symptoms, whereas Type II negative symptoms moderately improve, reflecting amphetamine-like activity from PCP (Balster 1986). Responses to an amphetamine (see discussion below) and PCP antagonist challenge test also differ between the groups: Type I patient's positive symptoms improve, while Type II patients do not respond. We postulate that individuals who manifest psychotic responses to PCP or PCP agonists have a biologic diathesis for sensitivity to PCP similar to that found in Type I schizophrenics, but with a diminished genotypic expression.

Three historical clinical observations have implicated PCP in the etiology of psychosis: the untoward psychiatric effects seen in approximately 10% of patients recovering from PCP-anesthesia (Greifenstein et al., 1958; Bums and Lemer 1981). the PCP-induced exacerbation of psychosis in schizophrenics (Luby et al. 1959). and PCP-induced psychosis in recreational drug users without history of psychosis (Fauman and Fauman 1980). We postulate that Type I schizophrenics would find PCP use aversive while Type II schizophrenics would abuse the drug for its amphetamine-like alleviation of negative symptoms. Based upon an early 1980's study of urine PCP levels in 1,454 psychiatric admittees to the West Los Angeles V.A. Medical Center, Brentwood Division (Gorelick and Wilkins 1986), we believe there is a bias of

dopamine disorder alters the tonal response of PCP receptors to the PCP ligand in predisposed individuals, resulting in psychosis.

PCP, Violence, and Stereotypy

Despite reports of violence associated with PCP use by humans (Fauman and Fauman 1980). a causal relationship between PCP and aggressive behavior remains unclear. We investigated the effects of varying doses of PCP on aggressive, affiliative, and individual behaviors in vervet monkeys (Wilkins et al. 1988; Dillon et al. 1987). Small doses of PCP attenuated affiliative behaviors, increased their liklihood of receiving aggression, but had no impact on locomotion. Low PCP doses caused dramatic grooming decreases, while aggressing and engaging in stereotypies were unaffected. At higher PCP doses, aggression and stereotypy obtained maximums substantially. above baseline values. When grooming was at its lowest level, aggression and stereotypies were at their maximum levels. Increased aggression was also noted in untreated cagemates. Loss of grooming and related affiliative behaviors appears to precede rapid development of aggression. The stepwise relationship between PCP-induced blockade of complex affiliative i.e. grooming) behaviors and development of extreme agonistic i.e. aggression) and stereotypic behaviors, indicates that social behavior protects against aggression. We propose that these PCP doses combined with amphetamines would produce stereotypy and aggressive behavior. If a small dose of PCP were given with amphetamine and neuroleptic, the affiiiative, aggressive, and stereotypic behaviors would be blocked in monkeys, and the analogous behaviors blocked in schizophrenics. Experimentally, we found that a larger PCP dose produced stereotypy and aggression in monkeys, without the need of supplementary amphetamine. Based upon known actions of dopamine blockers, we assume that neuroleptics would block the above behaviors.

Animal models exploring neurotransmitter functions in PCP-mediated behaviors may lead to development of new antipsychotic, antiviolence, and other types of psychotropic medication (See Balster, this monograph). Drugs that alter dopamine, serotonin, and sigma receptor activity appear to interact with behavioral actions of PCP. Dopaminergic system enhancement precedes PCP-induced stereotypy in rats (Hiramatsu et al., 1987; French and Vantini 1984). PCP is also thought to exert direct agonistic action on 5-HT2 receptors at medium to high doses and indirect agonistic action on 5-HT1 receptors at low and medium doses, as well as release met-enkephalin indirectly or directly by binding of PCP to sigma opioid receptors (Nabeshima, 1988). PCP also appears to activate central sigma systems with attendant locomotor activation in the rat (Iwamoto 1989). These models suggest that an ideal antipsychotic and antiviolence agent may be one that is a dopamine and/or serotonin antagonist and also blocks central sigma activity. In schizophrenics (see figure and earlier discussion) or subjects receiving PCP agonists, blood measures of neurotransmitter metabolites (e.g. homovanillic acid reflecting CNS dopamine, 5-hydroxy-indole acetic acid reflecting serotonin) and sigma activity in leucocytes (Wolfe 1988) may parallel the findings in animal studies.

PCP Induced Protracted Psychosis and Post Hallucinogen Perceptual Disorder

We propose that PCP-induced protracted psychosis and post-hallucinogen perceptual disorder are sequelae of recirculating PCP in individuals predisposed to psychosis. Non-ionized PCP is lipophilic and hence accumulates in fatty

some schizophrenic patients, compared to age/sex matched affective disordered patients, to abuse PCP, reflecting self-medication of negative symptoms (we did not dichotomize the subjects into Type I or Type II). Of 221 PCP positive subjects, 50 were schizophrenic (22.6%), while 13 were diagnosed with affective disorders (5.9%). Furthermore, 14.3% (50 of 350) of all schizophrenics were PCP positive, whereas only 8.0% (13 of 162) of all affective disordered individuals were positive. Though PCP positive patients in both groups tended to be young (54% of each group were between 25 and 34). schizophrenics were more likely to have high urine PCP concentrations (20% >100 ng/ml) than affective disordered patients (7.7% >100 ng/ml). The only diagnostic groups with greater rates of PCP use than schizophrenics were drug abusers and alcoholics. Our data is consistent with 4 previous studies (reviewed by Schneider and Siris 1987).

Select CNS actions of PCP. taken together with the dopamine hypothesis of schizophrenia (Haracz 1982) may explain a number of unresolved clinical phenomenon including: the wide spectrum of clinical presentation, the developmental nature of schizophrenic signs and symptoms, stress-related exacerbations of psychosis in stabilized schizophrenics, specific patterns of substance abuse, and the significant percentage of patients who are unresponsive to dopamine blocking neuroleptics. Haracz (1984) has postulated that long term potentiation (LTP) processes within the CNS control the timing of psychotic development in schizophrenics. PCP ligand activity may mediate LTP actions since 2-APV and 2-APH, drugs with PCP-like actions, are effective in altering LTP using the brief tetanic stimulation model (reviewed by Honore 1989). PCP ligand activity may also be involved in stress-induced psychotic exacerbations in schizophrenics. Pechnick et al. (1989) found that PCP doses as low as 0.5 mg/kg result in increased ACTH and corticosterone levels for up to 5 hours in rats. We postulate that stress results in increased endogenous PCP ligand levels in schizophrenic patients, leading to exacerbation of psychosis (esp. in Type 1 schizophrenics) and subsequent release of hypothalamic-pituitary-adrenal axis (HPA) stress hormones. This hypothesis is supported by recent in vitro findings (Su 1989) that endogenous steroid hormones, perhaps functioning in a feedback loop regulating PCP ligand levels, bind as a ligand to CNS sigma receptors.

Crow (1980) has proposed a division of schizophrenia into Type I (acute state) and Type II (defect or chronic state). Type I schizophrenia is believed to result from excessive dopamine activity, and is characterized by positive symptoms including delusions, hallucinations, thought disorder, normal computerized axial tomography (CAT) scans, and in general, a positive therapeutic response to neuroleptics. Type II schizophrenia is believed to result from structural CNS. deficit(s), and is characterized by negative symptoms including affective flattening and poverty of speech. abnormal CAT scans, and a generally negative therapeutic response to neuroleptics. Amphetamines worsen Type I symptoms but improve Type II symptoms. Cesarec and Nyman (1985) and Van Kammen and Boronow (1988) observed decreased negative symptoms in schizophrenics given amphetamines; these reductions were lost when amphetamine therapy was discontinued. Van Kammen et al. (1982). Angrist et al. (1985), and Lieberman et al. (1985.1987) report that schizophrenics exacerbating psychosis when given amphetamines are those patients who relapse sooner upon discontinuation of neuroleptic medication (e.g. Type I patients). Amphetamine administration upsets the mesolimbic (A10). and dopaminergic systems of Type I schizophrenics. These observations are consistent with French et al. (1988), who found that exogenously administered PCP alters A10 dopaminergic function (See French and Piercey, this monograph). We postulate that a

tissue (Misra et al., 1979). Lysis of fat may liberate PCP into the circulatory system, from where it is readily absorbed by brain. Washout of PCP from brain and other tissues is accomplished by acidifying tissues (e.g. patients are given cranberry juice, which contains the acidifying agent hippuric acid). We have also shown that acidification of body fluids results in significant decreases in blood-brain-barrier (BBB) uptake of PCP into the CNS (Wilkins 1986). Using the intracarotid single bolus injection technique (Oldendorf 1971), we found that as the injection mixture pH increases from 7.5 to 8.4, there was a modest 5% increase in the Brain Uptake Index (BUI) of PCP (65.8 + 5 to 70.8 + 5.6). Lowering the injection mixture pH from 7.5 to 6.8 resulted in a 36% decrease in BUI of PCP (65.8 + 5 to 42.4 + 1.5) (Student t test, $p < .01$). As the pH of the BBB capillary network is lowered, ionization of PCP increases and PCP uptake by brain decreases.

PCP Agonists and Neurologic Disorders

An optimal PCP agonist would help manage evolving stroke and early hypoglycemic shock, without attendant psychotomimetic effects. In both cases, patients manifest a dangerous and quickly changing clinical picture where a PCP agonist would block or attenuate ischaemic or hypoglycemic CNS damage while other stabilizing medical procedures are performed. Based on evidence that the NMDA receptor is central to epilepsy pathophysiology (Schwarz et al., 1987). we postulate that PCP agonists may also treat epilepsy or at least refractory epilepsy. We also speculate that PCP agonists may treat progressive CNS disease states, including AIDS-related dementia and dementia of the Alzheimer's type.

CNS Toxicity from PCP

We propose that PCP-induced neuropsychological deficits are secondary to CNS toxic effects of PCP agonists (Olney 1989). PCP induces neurotoxic effects on medium-to-large sized neurones in layers III and IV of the posterior cingulate and retrosplenial cortices, causing significant neuropsychological deficits in PCP abusers. In a study of PCP abusers, Lewis and Hordon found that 30% manipulated brain damage according to Trails B Test (a subtest of the Halstead-Reitan battery). Seventy percent were in the mild, moderate, or severe impairment range as determined by the Category Test, and a Mean Full Scale IQ of 92.5. These results may be complicated by polydrug abuse and aldulterated PCP. Neuropsychological deficits observed in PCP users are the result of PCP-mediated neurotoxic effects (Olney et al., 1989). We propose that studies of PCP abusers and patients receiving ketamine anesthesia will provide information on the risks of administering PCP agonists to humans.

Integration of PCP Actions with Crow's Type I and Type II Schizophrenia

Anticipated Results	Type I	Type II
Response to PCP agonist (small dose)	worsens pos. symptoms	No effect
Response to PCP agonist (larger dose)	worsens pos. symptoms	moderate improvement in negative sx (as amphetamine)
Response to PCP antagonist	improvement in pos. symptoms	no response
Ratio CSF & Blood HVA: CSF PCP ligand	High	Low
Positron Emission Tomography: ratio of dopamine to PCP/sigma receptor activity	High	Low
Drug abuse pattern	Tendency to avoid PCP	Abuse of PCP (as CNS stimulant)

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ACKNOWLEDGEMENTS

Geoffrey Smith and Brian Derrick performed the PCP assays. Leon Braun performed the BUI procedures.

AUTHORS

Jefferey N. Wilkins, M.D.
Chief of Clinical Psychopharmacology Unit
West Los Angeles VA Medical Center
Brentwood Division
and
Department of Psychology, UCLA

David A. Gorelick, M.D., Ph.D.
Department of Psychology, UCLA

Cannabinoid Modulation of Cyclic AMP. Accumulation in Synaptosomes

Patrick J. Little and Billy R. Martin

Δ^9-Tetrahydrocannabinol (Δ^9-THC) and other cannabinoids disrupt many membrane-bound enzyme systems. The precise role that this disruption may play in the pharmacology of the cannabinoids is unknown. Moreover, it is unclear whether these membrane effects represent a specific interaction with the enzyme molecule, or a non-specific change in activity of the enzyme due to perturbation of the lipid environment. One enzyme that has received much of the focus is adenylate cyclase, an integral membrane protein responsible for the formation of cyclic adenosine 3',5'- monophosphate (cAMP). The role of cAMP as a second messenger in most tissues including the CNS has been well documented. The ability of cannabinoids to alter adenylate cyclase activity has been best characterized in tissue culture, while these effects have not been as well characterized in brain preparations. The purpose of this investigation was to use structurally novel cannabinoids, which display potent cannabimimetic effects in mice, as probes to aid in further elucidation of the effects of cannabinoids on cAMP accumulation in the brain.

Male ICR mice were decapitated and their brains minus cerebellum were homogenized in 0.32M sucrose. A crude P_2 homogenate was layered on a discontinuous ficoll gradient to obtain synaptosomes. Synaptosomes were then maintained in a Krebs-Henseleit buffer and effects of cannabinoids on forskolin-stimulated increases in cAMP accumulation were determined. Levels of cAMP were determined using a competitive binding assay, with dextran-charcoal adsorption and centrifugation used to separate bound and free cAMP. All cannabinoids tested had little or no effect on basal cAMP levels, therefore the effects on forskolin-stimulated (either 0.1 or 1 μM increases in cAMP were determined. Δ^9-THC (1-100 nM) decreased cAMP levels by approximately 10%

following stimulation by 0.1 μM of forskolin, while concentrations of Δ^9-THC above 1 μM had little or no effect. CP-55,940 and CP-55,244 are structurally novel cannabinoids which possess very potent activity in mice. CP-55,940 significantly ($p < 0.05$) decreased cAMP levels in a concentration-dependent manner following 1 μM forskolin, with 1 μM decreasing levels by 15%. CP-56,667, the (+)-enantiomer of CP-55,940, inhibited forskolin-stimulated (1 μM) increases in cAMP by approximately the same level, however a concentration of 30 μM was required to observe similar degrees of inhibition. CP-55,244 significantly ($p < 0.05$) decreased the level of forskolin-stimulated (0.1 μM) cAMP accumulation between 0.03-10 μM with a maximal inhibition of approximately 20%. CP-55,243, the (+)-enantiomer had no effect on CAMP levels at concentrations as high as 30 μM. Unlike the other structurally novel cannabinoids, levonantradol enhanced forskolin-induced increases in cAMP by approximately 15% between 0.01-0.3 μM. Dextronantradol, the (+)-enantiomer also augmented the increase in cAMP caused by forskolin. Both levonantradol and dextronantradol decreased cAMP levels at concentrations above 1 μM.

Thus, there are some differences between the effects of the structurally novel cannabinoids and Δ^9-THC on cAMP accumulation in mouse synaptosomes, although they produce the same qualitative effects in vivo. Thus, the precise nature of the role that adenylate cyclase may play in the pharmacology of Δ^9-THC is unclear and needs to be more thoroughly investigated.

Supported by NIDA grant DA-03672 and NIDA Training grant DA-07027.

Affiliation: Department of Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298-0613.

Withdrawal From Benzodiazepine Dependence as a Discriminative Stimulus

M. W. Emmett-Oglesby and D. A. Mathis

Rats were maintained on a baseline of chlordiazepoxide (CDP), given in a nutrionally-balanced liquid diet, in two doses daily (100 mg/kg/day). Six-hours after the morning administration of CDP, subjects were trained to discriminate the benzodiazepine antagonist, flumazenil, 20 mg/kg, from saline using food reinforcement in a two-lever choice procedure. The discrimination was acquired rapidly, and lower doses of flumazenil substituted for the training stimulus with an ED50 of approximately 0.64 mg/kg. Cocaine, a drug with stimulus properties distinct both from CDP and withdrawal from CDP, was also tested. Rats selected the saline lever, indicating that the discrimination was based on an effect of flumazenil. In additional tests, the anxiogenic drug, pentylenetetrazole (PTZ), substituted for flumazenil. In contrast, rats trained to detect PTZ and tested with flumazenil alone did not substitute flumazenil for PTZ. However, if rats trained to detect PTZ were given this CDP diet for two weeks, flumazenil also substituted for PTZ. These data are consistent with the suggestion that subjects learned to discriminate withdrawal from CDP, and the data from tests with PTZ suggest that a predominant component of this discriminative stimulus is PTZ-like in character.

ACKNOWLEDGEMENTS: Supported by NIDA Grant DA RO1-3521.

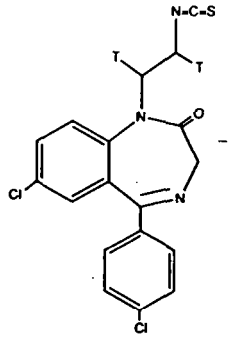
Affiliation: Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690

[^{3}H]AHN 086 Acylates Peripheral Benzodiazepine Receptors in the Rat Pineal Gland

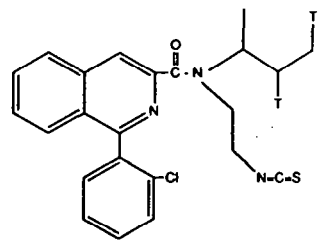
A. H. Newman, R. T. McCabe, J. A. Schoenheimer, P. Skolnick, K. C. Rice, J-A Reig and D. C. Klein

The peripheral-type benzodiazepine receptors (PBR), located in a variety of peripheral tissues and in the brain, have been characterized by two selective ligands, Ro 5-4864 and PK 11195. We have reported that PBR in rat kidney and heart are selectively acylated by AHN 086, an isothiocyanato-derivative of Ro 5-4864, and AHN 070, an isothiocyanato-derivative of PK 11195. These ligands have proven to be useful tools in the characterization of the PBR in these tissues. The synthesis of [^{3}H]AHN 086 (NEN, S.A. 45 Ci/mmol) has recently provided a high affinity, selective radioligand with which to characterize PBR. Rat pineal gland was chosen for this initial study since it has been shown to have a high density of PBR.

[^{3}H]AHN 086 was incorporated into rat pineal protein, bovine pineal protein and ovalbumin, however significant specific incorporation was only detected in the rat pineal. Gel filtration high performance liquid chromatography of digitonin-solubilized membranes from this tissue revealed a major peak of radioactivity with a retention time of 18.2 min corresponding to approximately 27 kDa. A major band corresponding to 30 kDa protein was labeled by t^{3}H]AHN 086 using sodium dodecyl sulfate-acrylamide gel electrophoresis (SDS-PAGE). The incorporation of [^{3}H]AHN 086 into this protein was inhibited by preincubation with the cols ligand. In addition, significant specific binding of [^{3}H]AHN 086 was not observed in bovine pineal gland and ovalbumin (tissues that do not have high affinity binding sites for Ro 5-4864).



[^{3}H]AHN 086



[^{3}H]AHN 070

The a parent molecular mass of PBR in rat pineal obtained with [^{3}H]AHN 086 is in good agreement with the target size of PBR estimated by radiation inactivation using [^{3}H]diazepam and by photoaffinity labeling with [^{3}H]flunitrazepam (both 1,4-benzodiazepines that bind to central and peripheral benzodiazepine receptors). In contrast, several studies using the isoquinolmecarboxamide [^{3}H]PK 14105 to photoaffinity label PBR in several different tissues obtained a molecular mass of approximately 18 kDa. These findings may indicate a multimeric arrangement of 18 and 30 kDa proteins in rodent tissue. We have now synthesized [^{3}H]AHN 070, and are presently characterizing its binding to PBR. Future studies with these two irreversible radioligands should prove valuable in determining the molecular arrangement, structure and function of PBR.

AUTHORS

Amy. Hauck Newman, Department of Applied Biochemistry, Walter Reed Army Institute of Research, Washington, D. C.

R. Tyler McCabe, Joyce A. Schoenheimer, Phil Skolnick, Laboratory of Neurosciences, NIDDK, NIH, Bethesda, MD

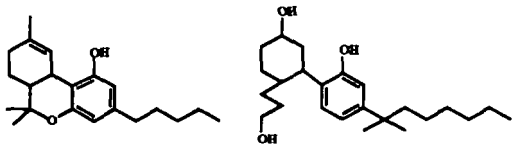
Kenner C. Rice, Laboratory of Medicinal Chemistry,- NIDDK, NIH Bethesda, MD

Juan-Antonio Reig, David C. Klein, Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD

Identification of Metabolites of CP-55,940 Formed In-Vitro by Mouse Livers

Brain F. Thomas and Billy R. Martin

A number of analogs of the basic cannabinoid molecule have been synthesized attempting to define structural requirements for analgesic activity. This research has produced potent analgesic agents such as (-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol (CP-55,940).



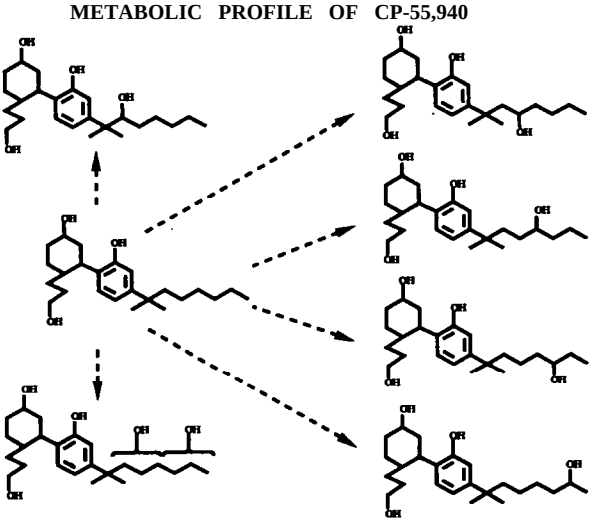
Δ^9- THC CP-55,940

The structural differences between the naturally occurring cannabinoid delta-9-tetrahydrocannabinol (Δ^9-THC), and this synthetic agent are quite dramatic, yet this compound has been shown to retain potent cannabimimetic activity in a number of behavioral tests. Due to the relatively recent development of this synthetic agent, its metabolism has yet to be thoroughly investigated. This study was undertaken to elucidate the primary metabolites of CP-55,940 formed in mouse liver preparations enriched with the appropriate cofactors for oxidative metabolism.

An S-9 supernatant prepared from the homogenate of 6 livers was enriched with 0.48 mg/ml NADPH, 0.16 mg/ml NADH, 0.4 mg/ml glucose-6-phosphate, and 1.0 µl/ml glucose-6-phosphate dehydrogenase prior to incubation with 2.0 mg of (X-55,940 and 3.0 µCi of ^{3}H-CP-55,940 at 37°C for 1 hr. The isolation of metabolites extracted with diethyl ether was performed using an HPLC equipped with a 25 cm C-8 reverse-phase column and a methanol/water gradient. The isolated metabolites were heated with BSTFA to form the ttimethylsilyl derivatives and, analyzed using a Hewlett-Packard 5988 Gas Chromatograph/Mass Spectrometer (GC/MS). Non-metabolized CP-55,940 was identified in the HPLC fraction collected at 33 min. The HPLC fractions collected between 16 and 18 min were pooled prior to derivatization and analysis by GC/MS. This sample was found to contain 5 monohydroxylated metabolites of CP-55,940 that could be separated on the gas chromatograph using an oven temperature ramp programmed at 10°C/min. The mass-spectral data obtained indicated that the 5 metabolites differed only with respect to the position of the hydroxylation on the dimethylheptyl side chain of CP-55,940. The HPLC fractions collected from 10 to 12 min were also pooled prior to GC/MS analysis. A number of compounds were detected in this sample that

have been tentatively identified as metabolites that are dihydroxylated on the side chain.

METABOLIC PROFILE OF CP-55,940



The determination that the side chain is extensively hydroxylated by the P450 mixed function oxidase system is interesting because it has been reported that metabolites of Δ^8-THC that are hydroxylated on the side chain retain some cannabinoid activity (Ohlsson et al. 1980). In addition the formation of the side chain hydroxyl group produces an-additional chiral center in the molecule. In support of these findings, the side chain hydroxylation reported to occur in this *in-vitro* preparation is consistent with the metabolic profiles of other cannabinoids (Harvey and Brown 1987).

Supported by NIDA grant #DA-03672 and NIDA training grant #DA-07027.

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AFFILIATION:

Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0613.

A New Analysis of Whole-Body Calorimetry Data

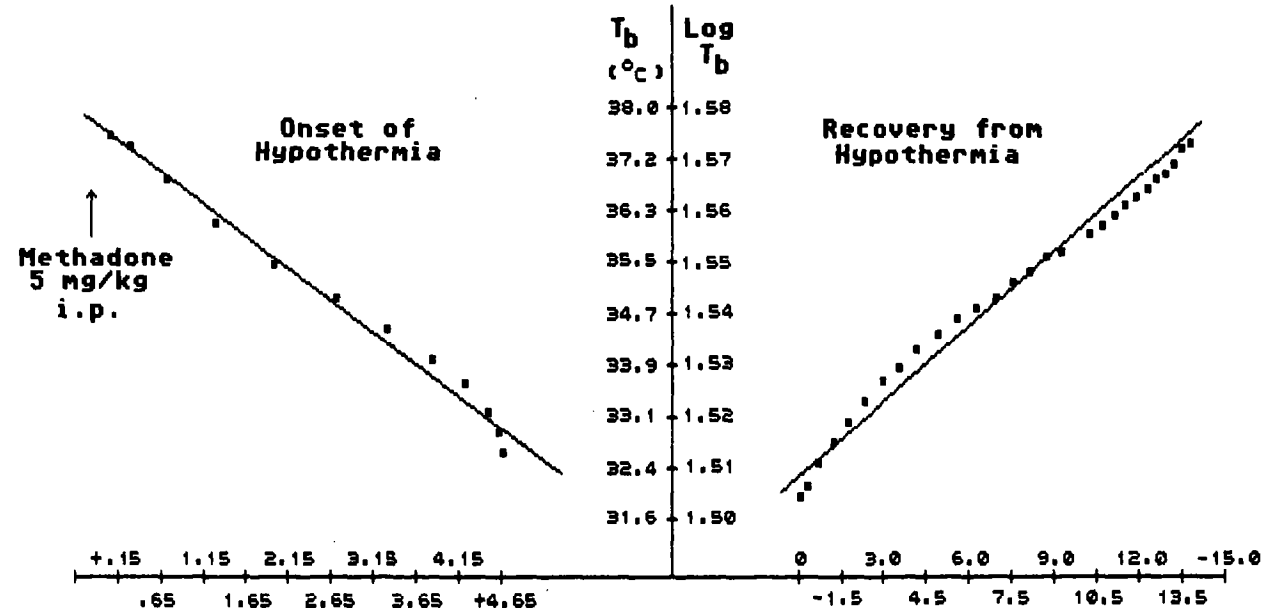
T. J. Lynch, R. P. Martinez, M. B. Furman
E. B. Geller and M. W. Adler

We have used gradient layer, whole-body calorimetry to study the mechanism by which mu and kappa opioid agonists induce hypothermia in rats. Continuous plots of drug-induced changes *in* body temperature (T_B), whole-body heat loss (Q) and oxygen consumption (VO_2) and the Q/VO_2 ratio were generated. Though simple enough, Q/VO_2, as related to ongoing changes in Tm, has not appeared in the literature. We have referred to it as *relative heat conservation* since it graphically depicts how morphine, methadone and U50,488H induce hypothermia despite sharp decreases in both VO_2 and heat loss (Lynch *et al.,* NIDA Res. Mono. 76, 1986). Regresslon analyses between log T_B and selected measures such as Q, VO_2 and Q/VO_2 yield correlation coefficients *in* the range 0.65 to 0.80. These are disappointingly low for such physical measures. Correlation of log T_B vs Q/VO_2 is poor because with the onset of drug-induced hypothermia, log T_B decreases continually, while Q/VO2 first increases but then decreases below baseilne. However, correlation between log T_B and the cumulative integral of the increase *In* $Q/V0_2$ ($\Sigma\Delta Q/VO_2$) yields a Pearson's I of 0.99 for the onset of hypothermia. During recovery from hypothermia, the same *r* value is obtained for the relation between log T_B and the cumulative integral of the decrease in Q/VO_2. The analysls reduces the four chart traces, T_B, Q, VO_2 and Q/VO_2 from an entire experiment to two straight lines, one for onset of, and another for recovery from opioid-induced hypothermia. The analysis proves that change in T_B is proportlonal to the integral of relative heat conservation, and that this can be quantltated without calculations based on the specific heat of rat tissue. We are hoping that the peak changes In Q/VO_2 caused by various opiolds, together with characteristic slopes of the regression equations (see figure) will tell us more about how agonists and antagonists affect different receptor populations. (Supported by NIDA Grants 5F32DA05300 and DA00376)

Affiliation: Temple University School of Medicine, Dept. of Pharmacology, Philadelphia, Pennsylvania 19140

METHADONE-INDUCED HYPOTHERMIA IN THE RAT

T_b = BRAIN CORTICAL TEMP Q/VO_2 = REL. HEAT CONSERVATION, CAL/ML O_2



Cumulative Integral of Q/VO_2 Increase

T_B =ANTILOG [-.01225 ($\Sigma\Delta Q/VO_2$)+1.5756]

r = -.995

Cumulative Integral of Q/VO_2 Decrease

T_B=ANTILOG [.00445 ($\Sigma\Delta Q/VO_2$)+1.5104]

r = .993

Novel [D-Pen2, D-Pen5]Enkephalin Derivatives With Increased Sigma Receptor Potency and Selectivity: Potential Tools for Opioid Pharmacology

Thomas F. Burks, Thomas H. Kramer, Peg Davis Gaza Toth and Victor J. Hruby

Improved definition of the central and peripheral functions of delta opioid receptors requires highly selective ligands. Presently, cyclic [D-Pen^2, D-Pen^5]enkephalin (DPDPE) is considered the most selective delta opioid agonist available for experimental use. It was our aim to develop new analogs of DPDPE with increased selectivity for delta vs. mu opioid receptors. Halogen (F,Cl,Br,I) substitutions were introduced in the para position of the Phe^4 residue of DPDPE. These compounds were evaluated in the mouse vas deferens (MVD) and guinea pig ileum longitudinal muscle/myenteric plexus (GPI) bioassay preparations for delta and mu potency (assessed by IC_{50} values) and for delta selectivity (assessed by the ratio of the IC_{50} in GPI to the IC_{50} obtained in MVD). Naloxone and selective antagonists, CTAP for mu receptors and ICI 174,864 for delta receptors, were employed to confirm specificity in each preparation. The compounds were evaluated in vivo for analgesic activity in the mouse hotplate test after intracerebroventricular (i.c.v.) and intrathecal (i.t.) administration.

All of the [p-halogenated-Phe^4]DPDPE analogs demonstrated improved potency and specificity for delta opioid receptors in comparison with DPDPE. In the MVD assay, [p-F-Phe^4]DPDPE was the most potent (IC_{50} = 0.84 nM) and [p-I-Phe^4]DPDPE was least potent (IC_{50} = 2.65 nM). In the GPI, [p-Cl-Phe^4]DPDPE was the most potent (4.8 μM) and [P-I-Phe^4]DPDPE was least potent (IC_{50} = 46.1 μM). The order of selectivity assessed by ratio of IC_{50} values for GPI/MVD was [P-I-Phe^4]DPDPE (17,374-fold selective) >[p-Br-Phe^4]DPDPE (8959-fold) > [p-F-Phe^4]DPDPE (5993-fold) > [p-Cl-Phe^4] DPDPE (5399-fold) > DPDPE (1336-fold). In MVD, all analogs and DPDPE were competitively antagonized

by ICI 174,864 but were unaffected by CTAP. Conversely, in GPI all were antagonized by CTAP indicating that effects in the GPI reflect actions at mu receptors. All of the analogs produced analgesia after either .i.c.v. or i.t. administration in mice. The A_{50} values were similar to those obtained with DPDPE, ranging from 0.3 to 6.0 nmoles/mouse i.c.v. and 3.3 to 18.2 nmoles/mouse i.t.

We conclude that additional conformational restriction induced by p-halogenation of the Phe^4 residue of DPDPE produced enhanced selectivity for delta opioid receptors. Further, analgesic potency of these peptides is associated with agonist actions at delta opioid receptors. These agents hold great potential as new tools for the study of the delta opioid receptor and its functions.

(Supported by USPHS grants NS19972, DA04248, DA02163, and DK36289.)

Affiliation: Departments of Pharmacology and Chemistry
The University of Arizona
Tucson, Arizona 85724

The Immune System and Morphine Dependence

N. Dafny, P. M. Dougherty and N. R. Pellis

Morphine dependence and the expression of withdrawal are dependent upon several discrete CNS regions. Ablation of these sites attenuates both the acute response to morphine as well as the expression of withdrawal (1). In addition, repeated administration of morphine directly to these areas induces a state of dependence identical to that observed following systemic injection of opiate (2). This syndrome has also been suggested to have an important immune component. An interaction between CNS opioid activity and lymphoid system has recently been suggested (3). Indeed ,there is significant overlap between many brain regions involved in the manifestation of opiate effects and those associated with immune system (IS) modulation (4). Yet, the participation of the IS in modulation central opioid actions has been underscored by the finding that various immunomodulating agents such as interferon (5), cyclosporin (6) and immune suppressive doses of gamma irradiation (7) attenuate the severity of naloxone-precipitated opiate abstinence in morphine-dependent rats. This has led to the hypothesis that an intact IS is essential for the expression of opiate withdrawal. This study tests this hypothesis by examining whether rats by adoptive transfer of splenic mononuclear cells will restore the withdrawal signs precipitated with naloxone in morphine-dependent rats.

Eighty-four male Fischer rats were used as donors of spleen cells. Cells from two donors were transferred (i.v.) to a single recipient (8). In some experiments splenic lymphoid cell populations were fractionated into plastic adherent and non-adherent subpopulations. Physical dependence upon morphine was induced by subcutaneous implantation of a pellet containing 75 mg of drug base for 72 hours. Five signs of naloxone precipitate withdrawal were scored. Wet dog shakes, teeth chatters, fecal boli, hyperactivity, scream to touch and diarrhea (3,5,6).

Adoptive transfer of 2-6 X 10^8 splenocytes to irradiated rats

(500 rads) prior to chronic morphine treatment restores the severity of all withdrawal signs precipitated by naloxone. In contrast, adoptive transfer of fractionated splenocyte subpopulations only partially restores withdrawal severity; and adoptive transfer of irradiated-splenocytes, red blood cells, or diluted number of normal splenocytes did not have any observed restorative effects (Fig. 1).

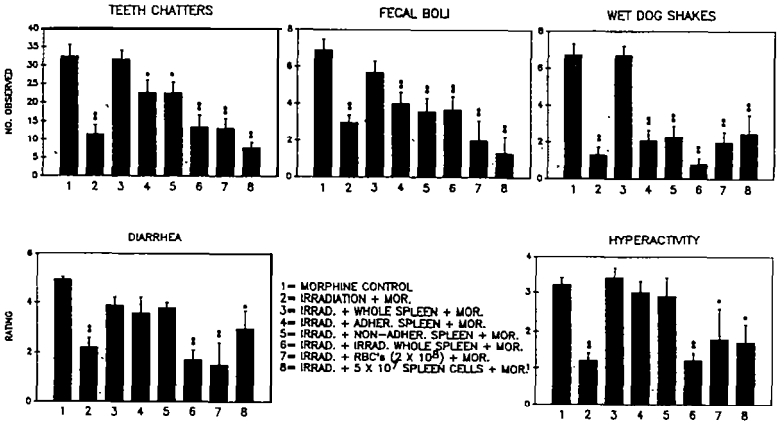


Figure 1. Summary of the signs of withdrawal observed for 10 min after 1.0 mg/kg naloxone (ip) injection.

The responses of animals to ionizing irradiation is rather conveniently stratified in a dose-dependent manner among the 3 major radio-sensitive organ system, the IS, the GI tract and the CNS. Exposure of animals to ionizing irradiation can at the appropriate dosages render them incapable of responding to antigenic stimulus without measurable changes in other body systems (9). Thus, irradiation was used to produce an animal model to test the effects of lymphoid organ ablation upon brain opioid activity. It was shown that irradiation reduce the severity of naloxone precipitated withdrawal (group 2) and transfer of splenic immune cell population from intact donors to irradiated recipients prior to chronic morphine treatment restored the severity of the withdrawal signs precipitated by naloxone (group 3). The control groups established that the adoptive transfer procedure itself does not explain the effects of immune reconstitution in counter-acting irradiation-induced withdrawal suppression. Moreover, the finding of a minimum cell numbers necessary for restoration of withdrawal, and the necessity of both splenocyte cell fractions for the full restoration supports the conclusion of a complex active participation of lymphoid cells in the manifestation of withdrawal. Whether the role played by the IS in opiate withdrawal is active or supportive remain to be resolved.

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Affiliation: University of Texas Medical School of Houston
Houston, Texas

Irreversible-Affinity Ligands for Mu Opioid Receptors

J. M. Bidlack, D. K. Frey, A. Seyed-Mozaffari and S. Archer

The morphine derivatives, 14 ß-(bromoacetamido)morphine (BAM), 14ß-(bromoacetamido)morphinone (BAMO), 14ß-(bromoacetamido)-7,8-dihydromorphine (H_2BAM), and 14ß-(bromoacetamido)-7,8-dihydromorphinone (H_2BAMO) were tested for their abiity to bind irreversibly to opioid binding sites in rat brain membranes. In the absence of a disulfide bond reducing. agent, these affinity Iigands bound only reversibly to opioid binding sites, The four ligands had receptor affinity and selectivity profiles similar to morphine. When membranes were incubated with dithiothreitol (DTT), followed by the affinity ligands, and then extensive washing, the affinity ligands inhibited the binding of the μ-selective peptide [^{3}H][D-Ala^2, (Me)Phe^4,Gly(ol)^5]enkephalin (DAGO) to membranes Under conditions when 90% of the [^{3}H]DAGO binding was inhibited, binding to δ and κ opioid binding sites was not altered by membrane alkylation with the affinity ligands, The irreversible inhibition of μ opioid binding was dependent on the concentrations of DTT and the affinity ligands. Opioids that bind to the μ opioid binding sites partially protected the μ binding site from alkylation, In addition to [^{3}H]DAGO binding, the binding of other radiolabeled opioids to μ binding sites was also inhibited by alkylation with the affinity ligands. The specificity of the alkylation is the *result* of DTT breaking a disulfide bond at or near μ opioid binding sites, This disulfide bond does not appear to be shared by δ or κ opioid binding sites. These studies demonstrate that upon reduction of a specific disulfide bond at or near the μ opioid binding site, 148 -bromoacetamido derivatives of morphine will akylate this site, resulting in the specific affinity labeling of μ opioid binding sites

ACKNOWLEDGEMENTS: Supported by grants DA03742 and DA01674 from the National Institute on Drug Abuse.

AFFILIATIONS: Department of Pharmacology, The University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642 and Department of Chemistry, Cogswell Laboratory, Rensselaer Polytechnic Institute, Troy, NY 12181

Differential Release of Substance P and Somatostatin in the Rat Spinal Cord in Response to Noxious Cold and Heat: Effect of Dynorphin A(1-17)

Paul J. Tiseo, Martin W. Adler and Lee-Yuan Liu-Chen

Dynorphin A, 1-17 (DYN), the proposed endogenous ligand for the *kappa* opioid receptor, has been reported to demonstrate no antinociceptive activity when tested in analgesic assays involving noxious heat (eg.. tail-flick and hot plate assays). Using a rat tail-flick analgesic assay that utilizes extreme cold as its noxious stimulus (an ethylene glycol/water mixture maintained at -10° C), we have reported a dose-related and naloxone-reversible antinociceptive effect for i.c.v. administered DYN. To investigate the mechanism of this antinociception, we designed a *push-pull* perfusion system which allowed us to measure changes in neuropeptide release in the spinal cord during exposure to noxious heat or cold. Male S-D rats were surgically implanted with two lengths of PE- 10 tubing inserted into the spinal subarachnoid space via the cisterna magna. At the time of testing, samples of CSF were collected both in the presence and absence of a noxious stimulus and substance P (SP) and somatostatin (SST) levels were then measured by RIA. Exposing the animal's tail to the noxious cold (30 sec/min for 20 min) resulted in a significant elevation in SP release (75% above baseline levels) but no change in the level of SST release. Conversely, exposure to noxious heat (50°C, 20 sec/min for 20 min) produced a significant increase in SST release (56% above baseline), but no change in the level of SP release. When DYN was administered i.c.v. just prior to noxious cold stimulation, there was a significant and dose-related (10-30 μg) inhibition of SP released in response to the stimulus. Naloxone (10 mg/kg, s.c.) was able to markedly reduce this inhibition. These results suggest that nociceptors specific for different types of thermal stimuli release different neurotransmitters in the dorsal horn, and that DYN produces antinociception to noxious cold, at least partly, by reducing the levels of SP released in response to the stimulus.

Temple University School of Medicine, Department of Pharmacology Philadelphia, PA.

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Naltrexone Discrimination in Morphine-Treated Monkeys

C. P. France and J. H. Woods

Opioid antagonist discrimination in morphine-treated subjects has been shown to be a useful method for studying opioid dependence and withdrawal in animals (e.g., France and Woods, 1987). In the present study, discriminative stimulus effects of opioid and nonopioid compounds were studied in three morphine-treated (1.78 or 3.2 mg/kg/day 3 hrs prior to experimental sessions) rhesus monkeys discriminating between subcutaneous injections of saline and 0.01 mg/kg of naltrexone (France and Woods, in press).

METHODS

Experimental sessions consisted of two or more discrete, 15-minute cycles. Monkeys responded under a fixed ratio 5 schedule of stimulus-shock termination; under this schedule brief electric shock was scheduled to be delivered every 15 sec in the presence of a distinctive stimulus (green lights). Monkeys could postpone schedule shocks and terminate the shock-associated stimulus for 30 sec by responding five times consecutively on the lever appropriate to the substance injected (subcutaneous) prior to that cycle (left lever, saline; right lever, 0.01 mg/kg naltrexone). Five-minute response periods alternated with 10-minute time out periods during which responses had no consequence and the shock schedule was not in effect. During test sessions five consecutive responses on either lever postponed scheduled shocks and increasing doses of drug were administered over consecutive cycles.

RESULTS AND DISCUSSION

Increasing doses of naltrexone produced a dose-related switch from the saline to the naltrexone lever with complete generalization occurring to doses of naltrexone larger than 0.0032 mg/kg. Other drugs with opioid antagonist actions (e.g., naloxone) substituted completely for naltrexone (i.e., produced an average of at least 80% responding on the naltrexone lever) and their potency as discriminative stimuli was similar to their reported potency for precipitating directly observable signs of withdrawal in morphine-dependent monkeys (Gmerek et al., 1987).

Doses of opioid antagonists that produced responding on the naltrexone lever did not markedly alter rates of lever pressing. A wide variety of opioid agonists as well as several nonopioids failed to substitute for the naltrexone discriminative stimulus up to doses that decreased markedly or eliminated lever pressing. Monkeys also responded on the naltrexone lever when they received saline, rather than morphine, 3 hrs prior to testing. Responding on the naltrexone lever, whether produced by administration of an antagonist or termination of morphine injections, was accompanied by signs that are characteristic of opioid withdrawal (irritability, miosis. salivation). Among the opioids and nonopioids studied, only compounds with morphine-like agonist actions under other conditions attenuated naltrexone-appropriate responding in morphine-abstinent monkeys (i.e., monkeys that had received saline rather than morphine 3 hrs prior to experimental sessions).

These data demonstrate the use of drug discrimination in morphine-treated monkeys and suggest dependence develops to single, daily injections of modest doses of morphine. The effects of opioid agonists, antagonists, and partial agonists under the conditions reported here are qualitatively similar to results obtained from observational studies in morphine-dependent monkeys (e.g., Gmerek et al., 1987). Drug discrimination, therefore, might provide an efficient, highly-quantitative alternative for studying opioid dependence in nonhuman primates.

In the coming year, it has been proposed by us to the Drug Evaluation Committee of the CPDD that compounds submitted to the CPDD for narcotic drug evaluation should be evaluated in both this procedure and in the observational procedures in morphine-dependent monkeys. Supported by USPHS Grants DA05018 and DA00254.

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AFFILIATION

Departments of Pharmacology and Psychology
University of Michigan, Ann Arbor, MI 48109-0626

Delta Opioid Receptor Selective Alkaloid Agonists and Antagonists

J. H. Woods, B. DeCosta, A. E. Jacobson, K. C. Rice, F. Medzihradsky, C. B. Smith, S. Comer, C. P. France and G. Winger

Assessment of the functional role(s) of the delta opioid receptor have been restricted by a lack of systemically active agonists and antagonists. Portoghese and colleagues (Portoghese et al., 1988) have recently synthesized a series of indole derivatives of naltrexone and oxymorphone that showed considerable delta selectivity in the guinea pig ileum and mouse vas deferens preparations. For example, naltrindole (Compd. 1, ibid. was 152 times more potent as a competitive antagonist of D-Ala^2,D-Leu^5-enkephalin (DALA) in the mouse vas deferens than of morphine in the guinea pig ileum. Methyl-naltrindole (Compd. 4, ibid.) was 8 times less potent as an antagonist of DALA than naltrindole in the mouse vas deferens, but showed no antagonist activity (at 200 nM) against morphine or ethylketazocine in the guinea pig ileum. Oxymorphindole (Compd. 8, ibid.), the N-methyl analogue of naltrindole inhibited the electrically-induced twitch in the vas deferens but not in the guinea pig ileum.

Our results confirm and extend those of Portoghese et al. (1988). We (Woods et al., this volume) found naltrindole to displace tritiated D-Pen^2,D-Pen^5-enkephalin (DPDPE) in rhesus monkey brain membranes with an EC50 of 0.21 nM compared to 9.53 nM against Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAGO) and 20.5 nM against U-69,593. Methyl-naltrindole had a very similar binding profile but had a slightly higher relative affinity for the mu receptor. Oxymorphindole displaced DPDPE with an EC50 of 3.37 nM compared to 1080 nM against DAGO and 688 nM against U-69,593.

In the mouse vas deferens, naltrindole appeared to be a delta-receptor selective, competitive antagonist. Up to 3 x 10^{-6} M naltrindole did not inhibit the twitch; the pA_2 for naltrindole was 9.44 against a delta agonist and 7.71 against a mu agonist. Although naltrindole (10^{-7} M) shifted the U-50,488 (kappa agonist) dose-effect curve slightly to the right, a pA_2 could not be determined because it also decreased the maximum response. Methyl-naltrindole had a similar profile of antagonist activity except a pA_2 could be calculated for methyl-naltrindole in combination with U50,488; it was 7.82. Oxymorphindole inhibited the twitch of the mouse vas deferens by 61%. Its inhibitory

actions were prevented by ICI 174864, a delta-receptor specific antagonist with a peptide structure. Since the effects of oxymorphindole were also antagonized by naltrexone, its partial inhibition might also have a mu-receptor component.

Oxymorphindole failed to exert thermal analgesic actions in rats up to 100 mg/kg (Comer et al., 1989) and exerted only a small analgesic effect in rhesus monkeys at a dose of 56 mg/kg. As other delta agonists have been shown to do, oxymorphindole antagonized the analgesic effects of morphine in rats; this effect was prevented by intracerebroventricular administration of ICI 174864. Oxymorphindole and naltrindole failed to substitute for the training stimulus in monkeys discriminating 1.0 mg/kg of codeine from saline. Up to a dose of 3.2 mg/kg naltrindole failed to antagonize the discriminative stimulus effects of the mu agonist alfentanil or the kappa agonist ethylketocyclazocine. Oxymorphindole also failed to maintain drug-injection responding in rhesus monkeys trained to self-inject alfentanil or cocaine. Taken together the results obtained with these compounds to date suggest the delta receptor mediates a different set of behavioral actions from those associated with either the mu or kappa receptor. (Research supported by USPHS DA 00254 and DA 05018.)

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AFFILIATIONS

Departments of Pharmacology (JHW, FM, CBS, CF and GW), Psychology (JHW, CF and SC) and Biological Chemistry (FM), University of Michigan, Ann Arbor, MI 48109 and Laboratory of Medicinal Chemistry, NIH-NIDDK, Bethesda, MD (BC, AEJ and KCR).

Biologically Active Conformer for Fentanyl and its Derivatives

Mark Froimowitz

In order to determine the three-dimensional structure responsible for its biological activity, MM2 calculations have been performed for fentanyl and its derivatives with a methyl group in the 3- and 4-position of the piperidine ring. lnitial calculations were performed on the (piperidine) N-methyl analogs of the compounds and the results for these are shown in the table. As with flexible 4-phenylpiperidines such as meperidine, ketobemidone, 3-demethylprodine, and atabicyclane opioids, fentanyl has equal populations of two mirror image conformers when the phenyl-containing 4-substituent is in the preferred equatorial conformation. Uslng a previously defined ligand model consistent with the general structure-activity relationships of phenyl-axial and phenyl-equatorial opioids, one conformer appears to be associated with typical morphine-like activity and the other with atypical activity including opioid antagonism. As in the more active enantiomer of ß-prodine, the cis-3-methyl In the more active enantiomer causes the molecule to favor the conformer associated with atypical activity. This is consistent with the atypical structure-activity relationships in the two compounds with regard to the effect of phenyl hydroxylation. In ß-prodine, the introduction of a phenyl meta hydroxyl appears to convert the compound from an agonist into an antagonist while hydroxylation of any position of either ring In fentanyl generally decreases the potency of the compound.

While it appears that fentanyl and its trans-3-methyl derivative act in phenyl equatorial conformers, phenyl axial conformers appear to be favorable for the cis-3-methyl derivative and those with an additional 4-substltuent. This may account for the 16 to 34-fold increase in potency in compounds with these substitutions.

As with previous calculations on phenazocine, the N-phenethyl group in fentanyl is quite flexible with small energy differences between conformers. However, based on constrained fentanyl analogs that retain activity, it appears that a trans dihedral angle is required for the ethyl group. This leaves two possible conformations of the N-phenethyl group due to the symmetry of the piperidine ring.

Table. Computed steric energies (kcal/mole) of (piperidine) N-methyl derivatives of fentanyl and analogs. Rotation is about the amide nitrogen-piperidine bond.

	phenyl-equatorial			phenyl-axial		
	60°	180°	300°	60°	180°	300°
fentanyl	2.8	1.6	1.6	8.7	4.6	4.6
cis-3-methyl	6.1	5.5	6.8	8.8	5.7	8.4
trans-3-methyl	2.7	2.3	-	10.9	7.0	7.3
4-methyl	8.2	7.1	7.1	13.5	6.9	6.9

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, Massachusetts 02178

A New Hypothesis on the Mechanism of Morphine's Effect on the Pupil

Thomas J. Lynch, Paul J. Tiseo and Martin W. Adler

We have formed a new view of how morphine (MS) induces pupillary fluctuations in the rat, based on studies using infrared video pupillometry, EEGs, respirometry, brain pO_2 measurement, kindling electrical stimuli and i.p. vs i.c.v. injection. In place of a presumed selective effect of MS (i.p.) on the Edinger-Westphal nuc. (EWN), we believe the primary effect of i.p. MS and the cause of miotic fluctuations is global intermittent electrographic bursting (IEB), propagating to and enslaving the EWN. Several types of data support this. First, after i.p. injection of MS, the first effect is most often an EEG burst of 5-20 min. duration. Second, IEB is global rather than limited to oculomotor structures. Therefore, it is highly unlikely that IEB starts in the EWN. Third. the onset of each miotic transient is time-locked with the beginning of each EEG burst, and the onset of each mydriatic transient is time-locked with the cessation of each burst. Fourth, respiratory slowing (10%. 5-10 sec. duration) occurs 3-5 seconds before all burst cessations and related mydriatic transients. This means that part of morphine's cyclic effect on the pupil occurs first in a respiratory center. Fifth, administration of an alerting stimulus (handclap) during MS-induced miosis causes immediate respiratory slowing followed by burst cessation and a mydriatic transient. The sequence of events during such arousal-induced conversion from burst-ON miosis to burst-OFF mydriasis is an exact replica of the normal transition which occurs during MS-induced pupillary fluctuations. This emphasizes the role therein of respiratory centers and, particularly, the reticular activating system, in which the former and the EWN are embedded. Sixth, EEG bursts induced by non-opioid means during MS-induced mydriasis also cause miotic fluctuation; miotic transients were time-locked to the onset of the kindling EEG after discharges in the cortex when elicited by stimulation of the amygdala. Similar effects were seen when IEB was elicited by a bicyclic organophosphate. These results indicate that burst propagation, even from distal structures, can be a key influence on parasympathetic outflow from the EWN. They also indicate that MS-induced miotic transients are not simply correlated with MS-induced EEG bursts but are caused by them. However, part of the old hypothesis remains intact, since we see a different response after i.c.v. injection. Instead of an initial EEG burst and miosis, mydriasis occurs slowly, without bursting. As drug effect continues, bursting and miotic fluctuations develop. Given i.c.v., it seems a direct effect of MS on EWN appears briefly, before bursting can be elicited in surrounding brainstem. As for the effect of i.p. MS on the pupil, we believe the original question of whey MS causes pupillary fluctuation should be rephrased as - "Why does MS cause IEB?"

AFFILIATION

Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

(Supported by NIDA Grant 5F32DA05300).

Intracerebroventricular (ICV) Clonidine Produces an Antianalgesic Effect Through Spinal Dynorphin A(1-17) Mediation

James M. Fujimoto and Kathleen Schaus Arts

Clonidine is thought to attenuate opiate withdrawal symptoms by acting on the locus coeruleus in a manner similar to morphine. On the other hand, Ossipov et al (1984) administered clonidine to the locus coeruleus and antagonized the analgesia produced by etorphine microinjected into the periaquaductal gray area. A number of reports indicate that clonidine administered into the brain produces an antianalgesic effect. We studied this antianalgesic effect in order to see whether it was similar to antianalgesia produced by physostigmine where spinal dynorphin A(1-17) may be an endogenous opioid mediating antianalgesia (Fujimoto and Rady, 1989).

ANTAGONISM OF MORPHINE BY CLONIDINE.

In male ICR mice, clonidine, 0.6 µg administered ICV at 10 min., antagonized the antinociceptive response (tail flick test, expressed as % MPE) to morphine administered intrathecally, IT, at 5 min. ED50 of morphine (log dose vs. probit %MPE) was increased from 0.21 µg (0.11-0.40, 95% Cl) to 0.83 (0.41-1.67). Next, dose (1.2 µg) and time (5 min.) of IT morphine was kept fixed while the dose of clonidine, ICV 10 min., was changed. Maximal antagonism occurred at 0.4 µg clonidine and as dose was raised up to 8 µg, effect became less: a biphasic effect occurred. Administration IT of naloxone (3 fg, 5 min) or nor-BNI (10 ng, 5 min) antagonized this antianalgesic action of clonidine on morphine analgesia.

NALOXONE, NOR-BNI UNCOVER ANAGESIA TO CLONIDINE.

By itself i.c.v. clonidine (dose up to 50 µg) did not produce analgesia. However, naloxone (5 fg) or nor-BNI (10 ng) given IT at 5 min. uncovered an analgesic action of clonidine, ICV 3 pg. This result along with those above indicated that clonidine had two effects: an overt antianalgesic and latent analgesic action. Because of the “paradoxical” actions of naloxone and nor-BNI, we proposed that the antianalgesic action of ICV clonidine was mediated spinally by an endogenous opioid which had antianalgesic actions.

DYNORPHIN A (1-17), ENDOGENOUS ANTIANALGESIC OPIOID?

Dynorphin A (1-17) appears to be this endogenous opioid. Dynorphin A (10 pg, 5 fmole) administered IT antagonized dose-dependently the analgesic action of morphine, IT 1 pg. In turn, this action of dynorphin A was antagonized by IT naloxone (5 fg) or nor-BNI (10 ng). Thus, antagonism of the action of dynorphin A and “paradoxical ” effects of naloxone and nor-BNI occurred at the same respective small doses. In addition, response to dynorphin A may involve receptors unique to dynorphin A because neither mu receptors (tested by DAMPGO) nor kappa receptors (tested by U50,488H) were blocked at the doses of naloxone (5 fg) and nor-BNI (10 ng) used.

Present results are consistent with a hypothesis that dynorphin A (1-17) may mediate a spinal antianalgesic system, a newly discovered function for dynorphin A(1-17), which can be activated supraspinally by clonidine or physostigmine. (Supported by the VA and NIDA 00451).

AFFILIATION: Research Service and Department of Pharmacology and Toxicology, VA Medical Center and Medical College of Wisconsin, Milwaukee, WI.

Characterization of Dynorphin A (1-17)-Induced Place Preference in Rats

Edgar T. Iwamoto

Certain drugs which are self-administered intravenously or which induce place-preferences in rats may do so by activating endogenous kappa-opioid systems. Young and Khazan (1983), Tang and Collins (1985), Steinfels et al. (1982) and Collins et al. (1984) showed that kappa opioids such as ethylketocyclazocine, ketocyclazocine, pentazocine, butorphanol, nalbuphine and nalorphine are self-administered intravenously by rats. The fact that these drugs are kappa agonists with partial-agonistic or full antagonist activity at the mu-opioid receptor suggests that central kappa receptor stimulation may be a determinant of drug dependence. This premise is further substantiated by the work of Khazan and collaborators who reported that dynorphin$_{1-13}$ at doses of 125 and 250 ug/kg/injection was self-administered intravenously by rats (Khazan et al., 1983).

Recently we reported that one intracerebroventricular injection of 2.3 or 3.5 nmol of dynorphin$_{1-17}$ induced a significant preference response in rats in the place-conditioning paradigm (Iwamoto, 1988). Place preferences were not induced by two probable metabolites of dynorphin$_{1-17}$, [leu^5]-enkephalin and des-tyrosine dynorphin A$_{2-17}$; the latter peptide does not bind to opioid receptors (Walker et al., 1982; Young et al., 1987). A 10-fold excess of naloxone was necessary to antagonize dynorphin preferences; these data indicated that kappa receptors may be important determinants of dynorphin-induced place conditioning. Stevens et al. (1988) reported that rats self-administered dynorphin$_{1-17}$ into the the CA3 region of the dorsal hippocampus. The purpose of the present experiments was to examine the dorsal hippocampus as a site of action of dynorphin-induced place conditioning, and to characterize dynorphin$_{1-17}$- induced conditioned place preferences using dynorphin congeners and nor-binaltorphimine, a selective kappa receptor antagonist (Portoghes et al., 1987).

Sprague-Dawley male rats were implanted unilaterally with a guide cannula aimed at the dorsal hippocampus. After one week, animals received either 'treatment' (peptide in 500 nl of phosphate buffer or 500 nl of buffer alone) or 'no treatment' (no injections) before being placed in the black or white chamber of a three-chambered

place-conditioning apparatus (Iwamoto, 1986) for 20 min; the next day the rats received the opposite treatment and were placed in the opposite chamber. On Day 3, the animal was started in the middle gray chamber and given free-access to the entire place-conditioning apparatus for 15 min. The number of sec spent in the treatment-paired (T) and the non-treatment-paired (NT) chambers was recorded. A preference ratio, PR, was calculated as [T-NT]/[T+NT] for each rat. As an example, if T = 450 sec and NT = 200 (with the remaining 250 sec being spent in the middle gray chamber), then PR = +0.38.

One dorsal hippocampal injection of 1 nmol of dynorphin,.,, (DYNA) conditioned a significant (Tukey's test) place preference response: DYNA PR = 0.38 ± .07 versus buffer treatment, PR = 0.02 ± .07. Two nmol of nor-binaltorphimine coadministered with the 1 nmol of DYNA completely blocked the DYNA preference response, PR = 0.09 ± .05. Nor-binaltorphimine alone was inactive in the place conditioning paradigm, PR = 0.04 ± .05 (N = 12 for all groups). In other experiments, significant place preferences were also conditioned by single, dorsal hippocampal injections of 1 nmol of dynorphin$_{1-17}$ amide (PR = 0.37 ± .lO), 1 nmol of dynorphin B (PR = 0.33 ± .11 and 1 nmol of alpha-neo-endorphin (PR = 0.34 ± .lO); the control PR = - 0.02 ± .06. The following peptides were inactive in the dorsal hippocampus (control PR = 0.01 ± 0.04): 1 nmol of dynorphin,.,, (PR = 0.10 ± 0.03), 1 nmol of dynorphin (Experiment 1, PR = - 0.11 ± .07; experiment 2, PR = 0.08 ± .07), 2 nmol of dynorphin$_{1-8}$ (PR = 0.06 ± .14) and 1 nmol of rat beta-endorphin (PR = 0.06 ± .08). The caudate nucleus was a negative site; 2 nmol of DYNA was inactive.

In a separate experiment, subcutaneously administered nalorphine was used as the conditioning drug. On Days 1,2,4,6,7 and 9, buffer or 1.25, 2.5 and 5 mg/kg of nalorphine hydrochloride was administered SC immediately before place conditioning for 20 min. On Days 3,5 and 8, all animals were injected with buffer before being placed in the opposite chamber for the 20 min conditioning session. Testing was performed on Day 10. Nalorphine induced place preference responses in a dose-related manner (control PR = - 0.13 ± .16; 1.25 mg/kg PR = 0.07 ± .08; 2.5 mg/kg PR = 0.12 ± .07; and 5 mg/kg PR = 0.26 ± 0.07, N = 5 per group. Significant slope of the regression, P = .002). The PR for the 5 mg/kg group was significantly different from control, P < .05, Tukey's studentized range test.

The present data suggest that DYNA-induced place preference in rats is mediated at kappa receptors in the dorsal hippocampus, that activity resides in the full-length, 17 amino acid peptide, and that the effect is mimicked only by certain prodynorphin products. Since there is little evidence that nalorphine is a partial mu-opioid agonist, the results of the SC-administered nalorphine conditioning experiment support the hypothesis that some opioid alkaloids can condition place preferences by stimulating kappa receptors.

A complete list of references may be obtained from the author.

Department of Pharmacology, MS-321
University of Kentucky College of Medicine
Lexington, KY 40536-0084

Evidence for Rapid Development and Loss of Opioid Tolerance to Fentanyl in the Rat

Thomas H. Kramer, Elizabeth A Ayres, and Thomas F. Burks

We are seeking to improve our understanding of the in vivo potency of opioid drugs and the phenomenon of tolerance by studying relationships between drug concentration and effect in intact animals. By correlating effect with concentration, rather than dose, we hope to control for the influence of drug disposition on apparent pharmacologic effect. These studies represent our initial experience with simultaneous assessment of pharmacokinetics and pharmacologic effect.

METHODS

We measured the antinociceptive actions of the synthetic μ opioid fentanyl in male Sprague-Dawley rats. Jugular venous cannulae were implanted under anesthesia three days prior to study. On the day of testing, fentanyl citrate (30 or 100 μg/kg) was injected subcutaneously, intraperitoneally, or intravenously in a single dose. Blood samples were obtained frequently after the dose, and the plasma separated and frozen. Antinociception was measured using a standard 55° C hotplate. Latency (in seconds) to jumping or hindpaw licking was recorded before and frequently throughout the experimental sessions. Maximum allowable latency was 45 sec. Fentanyl concentrations were measured by radioimmunoassay (FEN-RIA 200, Janssen Life Sciences). Controls included rats which underwent identical preparation and testing but received saline injections rather than fentanyl (Control 1, n=4), and rats treated with fentanyl and tested for antinociception, but not subjected to surgery or blood sampling (Control 2, n=5). Hematocrit was determined periodically in the first control group. Fentanyl concentration vs. time data were fit to[1] standard pharmacokinetic equations by weighted nonlinear least squares regression[1]. Hotplate latencies were plotted against time and concentration and evaluated for each individual-rat.

RESULTS

Mean control latency (± SD) was 11.3 ± 2.7 sec. The onset of antinociceptive effect was extremely rapid after fentanyl administration. Intravenous fentanyl produced maximal latency (45 sec) within 30 sec of dosing; subcutaneous or intraperitoneal dosing demonstrated a delay of several minutes before onset of apparent effect. Maximal latency was achieved in 18 of the 20 rats which received fentanyl. Non-treated control rats demonstrated no trend to increasing or decreasing latency over the study period. Though hematocrit was decreased as much as approximately 40% at the end of the studies, this was not associated with a change in nociceptive responses.

Fentanyl-treated animals showed distinctly biphasic antinociceptive responses, with increases in hotplate latencies occurring subsequent to loss of the original antinociceptive effect. The temporal pattern of the peaks and valleys in the effect vs. time profile was different for each animal, such that taking the mean latency of the

group at each time point obscured the pattern present in the individuals. The increases and decreases in latency progressed in a systematic fashion and could not be accounted for by random variation in response or imprecision in measurement. Mean peak latency (first peak, ±SD) was 43.3 ± 4.0 sec; mean "valley" latency was 12.6 ± 8.4 sec; mean second peak latency was 28.4 ± 11.5 sec. Control and "valley" latencies were not significantly different; mean latencies at peak 1 and peak 2 were both different from control and valley latencies, and from each other (all $p<0.01$, ANOVA followed by Neuman-Keuls test).

Fentanyl pharmacokinetics were unremarkable in these rats. All concentration data sets were best fit to biexponential pharmacokinetic equations with bolus, zero-order, or first-order drug input for the intravenous, subcutaneous, or intraperitoneal routes of administration, respectively. Fentanyl elimination appeared linear and independent of dose. Mean (±SD) distribution and eljmination rate constants (α and β) were 0.058 ± 0.025 min^{-1} and 0.0074 ± 0.0041 min^{-1}, respectively. Importantly, no late increases in concentration, associated with increased antinociception or otherwise, were noted.

DISCUSSION

Several potential explanations for recurrent antinociceptive responses should be considered. An effect of blood loss or learning over the course of the experiment is ruled out by the stable responses in control group I. Late increases in total fentanyl concentration, caused by enterohepatic recirculation of drug, for example, are not present as demonstrated by the linear and non-complex pharmacokinetics observed. Late increases in free, but not total, fentanyl concentration might be caused by the progressive loss of plasma proteins which bind fentanyl, due to blood sampling. However, animals in control group 2, which did not undergo sampling, still demonstrated biphasic antinociceptive responses to fentanyl.

Formation of an active metabolite of fentanyl might also explain these results, however, little or no evidence is available to support the existence of a fentanyl metabolite with opioid agonist activity. Fentanyl metabolism has been characterized in the rat; the principle fentanyl metabolite is a des-phenethyl derivative[2,3] which should not have appreciable opioid receptor affinity. Other metabolites (present in much smaller quantities[2,3]) would need to possess extraordinary potencies to produce the effects noted here after a single dose.

A final possible cause of these results is rapid desensitization to the effects of high fentanyl concentrations. The early concentrations measured in these studies are far in excess of those needed to produce antinociception in our paradigm. It is possible that these high concentrations rapidly induce a tolerant state. As redistribution of fentanyl out of the brain is extremely rapid[4], reduction in brain fentanyl to more modest levels could occur faster than resensitixation to the effect of fentanyl. This would result in loss of antinociceptive effect, followed by return of effect as resensitization occurred in the post-distributive phase of fentanyl elimination.

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AFFILIATION

Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ 85724.

Supported by USPHS Grant DA02163.

Inpatient vs. Outpatient Cocaine Abuse Treatments

Thomas R. Kosten, Bruce J. Rounsaville and Susan H. Foley

Two widely used treatments for cocaine abuse are brief (21-28 day) inpatient hospitalizations and outpatient psychotherapy. While some studies have suggested that one year outcome for these two forms of treatment may be equivalent for alcoholics, these two treatments have not been compared for cocaine abusers. Furthermore, comparisons of comorbid psychopathology in these patients at intake to the two types of programs have been incomplete. We have therefore compared these two treatments in cocaine abusers who were carefully diagnosed using DSM-III-R. Preliminary results in 303 treatment seeking cocaine abusers (153 outpatients - OP and 150 inpatients - IP) indicate that they are predominantly male (66% OP vs 73% IP), white (58% OP vs 69% IP), unmarried (72% OP vs 70% IP), young adults (29 ± 0.6 years OP vs 27 ± 0.5 years IP). In comparing the IP to the OP cocaine abusers on rates of comorbid psychopathology and severity of cocaine abuse several differences emerged. The IP cocaine abusers reported more days of cocaine use over the month before admission to treatment (17 ± 2 days vs 10 ± 1 days) and more years of abuse (4.5 ± 1 years vs 2.5 ± 0.5 years). Rates of psychopathology showed more antisocial personality in OP (37% vs 29%) and more attention deficit disorder in IP (25% vs 40%). Depression and anxiety disorders showed significant gender effects with females having higher rates of depression in IP than OP (35% vs 18%). In contrast, rates of depression *were* higher in OP males than in IP males (22% vs 35%), and there were no differences in anxiety. The onset of the anxiety, which were mostly phobias, and attention deficit disorders generally preceded the cocaine abuse (90% and 99%, respectively), but the depressive disorders and alcoholism both had their onset most frequently after cocaine abuse (65% vs

80%), respectively). Overall, the IP cocaine abusers had more severe cocaine abuse, past suicidality and current depression, particularly in females and more attention deficit disorder in the younger males (< 30 years old). This increased psychopathology and severity of cocaine abuse may lead to the need for hospitalization.

Department of Psychiatry
Yale University School of Medicine
New Haven, Connecticut

Outcomes of Cocaine-Dependence Treatment

Forest Tennant

Little is known about treatment outcomes of cocaine dependence or its natural epidemiologic course. The author has been able to track 67 cocaine-dependent athletes from four different sports. They were identified between 1983 and 1988. and they have been followed for periods ranging from 4 to 60 months (mean 19.3). At the time of identification, 25 (37.3%) were treated by standard, inpatient hospitalization and 42 (62.7%) were treated as outpatients. During follow-up. these patients submitted a total of 2,484 observed urine specimens which were analyzed for alcohol and metabolites of cocaine, and marijuana. A total of 41 (60.3%) suffered relapses which required some form of retreatment. Neither inpatient nor outpatient treatment appeared to influence outcome since 16 (64.0%) of hospitalized versus 25 (59.5%) of non-hospitalized cases required retreatment (PNS).

During the 1980's cocaine addicts have increasingly sought medical detoxification. We have attempted to develop a low cost, outpatient procedure which can be utilized in our system of clinics throughout Southern California. The medical regimen is a step-wise procedure which utilizes amantadine, levo-dopa, bromocriptine, desipramine, and prazosin as well as ammo acid supplements. Patients attend the clinics daily until their urine is negative as determined by immunoassay. Between 1985 and 1988, three separate pilot studies were done in West Covina, California to determine the feasibility of this approach. Outcomes indicate that over 50% can be detoxified by this method (See Table).

The major conclusion to date is that low cost. outpatient treatment of cocaine dependence with dopamine agonists, amino acid supplements. and antidepressants is an effective detoxification procedure in about one-half of the cases. We do not have long-term follow-up data on relapse rates in this group. Despite some success in treating cocaine addiction, there is an obvious need for a more effective medical treatment.

COCAINE ADDICTS TREATMENT OUTCOMES				
Study No.	**Dates**	**No.**	**Urine End-Point***	**No. Succesful Treatment**
1	Oct. '85 -Aug. '86	77	EMIT 300 ng/ml	37 (57.8%)
2	Sept. '86 - April '87	29	EMIT 300 ng/ml	16 (66.7%)
3	Jan. '88 -Dec. '88	71	PFI 30 ng/ml	37 (52.1%)

• EMIT is Enzyme Immunoassay with a urine testing sensitivity of 300 ng/ml and PFI is Polarized Fluorescent Immunoassay with a sensitivity of 30 ng/ml.

AFFILIATIONS:

Community Health Projects Medical Group, 336½ South Glendora Avenue. West Covina, California 91790

and

UCLA School of Public Health, Los Angeles, California 90024

Carbamazepine Treatment of Cocaine Dependence in Methadone Maintenance Patients with Dual Opiate-Cocaine Addiction

Kenneth L. Kuhn, James A. Halikas and Kenneth D. Kemp

ABSTRACT

The cocaine epidemic of the past decade is undermining the efficacy of methadone maintenance (MM) treatment. In an open clinical trial, 12 MM patients addicted to cocaine were offered carbamazepine (CBZ) as a pharmacological adjunct in the treatment of cocaine dependence. Patients reported mild opiate withdrawal symptoms after initiation of CBZ treatment. Methadone serum trough (MST) levels taken before CBZ were compared with levels taken 7-10 days later, with an average decrease in *MST* levels of 60%. Patients (6) who continued on CBZ (average 144 days) reported a reduction in cocaine use, documented by random urine drug screens. Non-completers (6) who did not continue on CBZ (average 17 days) had no change in cocaine use.

METHODOLOGY

The 12 MM patients were intravenous cocaine users, who averaged 86 cocaine using days per 100 days prior to referral to the cocaine clinic. The average amount of cocaine use was 3.5 grams weekly; average cocaine use history was ten years; history of illicit drug use was 22 years. Of the 12, three were female, and of the nine males, four *were* black. Their average age was 41. They averaged 11 lifetime drug related arrests and four prior treatments. All punitive sanctions for *drug use were* suspended during participating in this program.

CLINICAL OBSERVATIONS

Four patients began CBZ treatment as inpatients on the chemical dependency unit at the University of Minnesota. MM dose range was 65-80 mg, given as a morning dose. Because of intense craving in the first two patients, CBZ dose was increased to 800-1200 mg/day, given in two equal doses. Within 36-48 hours both patients complained of restlessness and insomnia which was progressive during the night, and was relieved by the next morning MM dose. An opiate withdrawal rating scale documented objective criteria for mild methadone withdrawal, with symptoms reappear-

ing 12-16 hours after the MM dose. This suggested the possibility that CBZ had lowered the steady-state equilibrium of the methadone. Urine drug screens were negative for other hepatic enzyme inducing drugs. MST levels were below 80ng/ml, with a range of 61-79ng/ml.

Eight patients began CBZ as outpatients, with weekly prescriptions given to six and daily monitored dosing in two. Pre-CBZ MST levels were above 120ng/ml with a range of 121-179 ng/ml. Outpatients were started on lower CBZ doses, with 100mg taken three times daily as the most frequently used schedule. Compliance was determined by CBZ serum levels and random urine screens. Post-CBZ MST levels taken 7-10 days after starting CBZ decreased by an average of 60%. MM dose increases of 10mg were recommended when MST levels were below 80ng/ml. Patients who were given weekly prescriptions had more difficulty stabilizing on the medication compared with the two monitored patients.

In addition to the pharmacologic therapy, a two hour weekly support group was offered. The group provided HIV-l prevention and chemical health education, relapse prevention, peer support and a review of traditional recovery issues. Patients openly shared their concern regarding the possible effect of CBZ on methadone metabolism, which reduced compliance in the study.

RESULTS

The six MM patients who took CBZ (average 144 medication days) reduced their frequency of cocaine use from 65/100 days pre-treatment to 23/100 day post-CBZ therapy, an overall change of 65%. Non-completers (6) reduced their overall cocaine use by less than 20%. While improved outcome of the CBZ completer group may be a measure of program compliance rather than pharmacologic effect of CBZ, it merits further investigation.

DISCUSSION

CBZ is known to stimulate the P450 microsomal hepatic enzymes, resulting in decreased steady-state levels of phenytoin, primidone, doxycycline, clonazepam and other medication metabolized by this common pathway in the liver. However, its specific effect on methadone has never been studied pharmacokinetically. This finding is being investigated in a controlled setting.

Although the use of CBZ in MM patients requires a dosage adjustment in the methadone, it may prove to be a useful adjunct in the treatment of cocaine dependence in this population.

AUTHORS

Kenneth L. Kuhn, M.D., Fellow in Chemical Dependency
James A. Halikas, M.D., Professor of Psychiatry and CoDirector of the Chemical Dependency Treatment Program
Kenneth D. Kemp, M.D., Fellow in Chemical Dependency, University of Minnesota, Box 393, Minneapolis, MN 55455

Quantitative Urine Screening for the Diagnosis and Treatment of Cocaine Abuse

Forest Tennant

Recently, radioimmunoassay (RIA) and polarized fluorescent immunoassay (PFI) screening techniques have been developed which have sensitivity levels below the usual qualitative screening techniques, and the ability to quantitate drugs of abuse within specific ranges. Quantitative results are very compatible to those obtained by gas chromatography/mass spectrometry. Additionally, these techniques can rapidly determine urine concentrations and have a low enough cost to be routinely utilized in a clinical setting. Since introduction of the PFI technology to the commercial market in 1987, we have utilized it with several hundred patients to assist with the diagnosis and treatment of drug abuse. Reported here is a series of 138 cocaine users. One group of 67 patients were referred for counseling and urine testing, and who reported occasional use of cocaine ranging from 1 to 3 times per week. The other group of 71 atients claimed to be addicted and voluntarily entered an outpatient detoxification program. Urine specimens collected from patients. on the day of admission, revealed that urine concentrations of cocaine metabolites were significantly different in the two groups.

Admission urines contained cocaine metabolite in 54 (76.1%) of the 7 1 addicted patients and in 24 (35.8%) of the occasional users. The 17 addicted patients who showed no cocaine metabolite in urine on the day of admission reported that they had ceased cocaine administration 4 to 16 days prior to this time, but they were experiencing so many withdrawal symptoms that they desired medical treatment. Only 3 of the 67 (4.5%) occasional users compared to 40 (56.3%) of the addicted patients showed cocaine metabolite in their urine on admission at a level of 1000 ng/ml or above (x^2 = 24.24; df = 1; P<.005). Two of the three occasional users who demonstrated over 1000 ng/ml on admission were teenagers who immediately, after the test, informed their parents that they were using cocaine daily. They were consequently transferred to detoxification treatment. The third patient submitted two urine specimens within the following two weeks which did not contain cocaine metabolite. Twenty-nine (40.8%) of the addicted, compared to 2 (3.0%) of the occasional users, demonstrated 5000 or greater ng/ml concentration on admission (x^2 = 77.19; df = 1; P<.005). Sixteen (16) of 24 (66.7%) occasional, compared to 10 of 54 (18.5%) addicted patients who had cocaine metabolite in urine on admission had concentrations less than 300 ng/ml (x^2 = 17.06; df = 1; P<.005). The mean urine concentration of addicted and compared to occasional users was 2435.2 ± 2298.1 S.D. (Range 40-5000) and 192.7 ± 673.8 S.D. ng/ml (Range 30-5000) respectively

(t = 45.43; df = 136; P<.005). The mean urine concentrations among the 54 addicted patients and 24 occasional users who submitted a positive urine on admission were 3201.9 ± 2115.3 S.D. (Range 40 - 5000) and 537.9±1053.1 S.D. ng/ml (Range 30-5000) (t = 25.12; df= 76; P<.005).

Although there was overlap in urine concentrations between the occasional and addicted users, data from these subjects indicate that it is frequently possible to determine if a drug user is likely addicted, or an occasional user. Even in questionable cases, repetitive quantitative testing over a few days time will likely clarify the question, since occasional users usually clear their urine withing 2 to 3 days. In addition to a significant difference between the admission urine concentrations of addicted and occasional users, high drug concentrations in the addicted users was often associated with a poorer treatment outcome. Twenty (20) of 31 (64.5%) addicted users, with an admission urine concentration under 1000 ng/ml comared to 17 of 40 (42.5%) with urine concentration over 1000 ng/ml, were able to detoxify and leave treatment with a negative urine (P<.005). Some addicted patients did not appear to be able to totally clear their urine of cocaine metabolite for as long as two to three weeks after they reported cessation of cocaine use. Other recent studies also document that cocaine addicts may not clear their urine for several days after last use.

Clearly, an attempt to evaluate treatment efficacy of cocaine addiction should, in great part, be based on the treatments' ability to eliminate all cocaine metabohtes from urine as determined by sensitive, quantitative testing. The receptivity of quantitative urine testing with patients and third parties has been gratifying. Practitioners are encouraged to utilize quantitative urine testing to enhance the diagnosis and treatment of cocaine abuse.

AFFILIATIONS:

Community Health Projects Medical Group, 336½ South Glendora Avenue, West Covina, California 91790
and
UCLA School of Public Health, Los Angeles, California 90024

Urine Testing During Treatment of Cocaine Dependence

William M. Burke, Narispur V. Ravi, Vasant Dhopesh, Barry Vandegrift, Iradj Maany and A. Thomas McLellan

During the past decade the use of cocaine has reached epidemic proportions in the United States. As a result of increased availability and relative decrease in price cocaine has become a major health problem in all segments of society. A number of treatment programs that have been designed to address this problem include urine monitoring as part of the therapeutic approach. There are, however, indications that the current literature may underestimate the length of time that metabolites can be detected in the urine after the use of large amounts of cocaine.

When using urine monitoring, misinterpretation of results may lead to inappropriate interventions that could weaken the therapeutic alliance as well as decrease the value of testing in the eyes of both the patient and the therapist. This may be particularly true with the treatment of cocaine dependence where lapses and relapses are frequent occurrences in the natural history of the disease and it becomes important to distinguish single use from repeated use. Until recently the literature suggested that benzoylecgonine could be detected in the urine for only 72 hours after last use of cocaine . This is the current standard of practice for interpreting results. However, there is an anecdotal report in the toxicology literature of one patient excreting low levels of benzoylecgonine for 15 days and Weiss and Gawin have recently reported three cases of protracted elimination of cocaine metabolites in high-dose cocaine abusers. Their study found that benzoylecgonine could be detected at levels of 300ng/ml or above for 10 - 22 days after cessation of cocaine intake and at that level, without subsequent cocaine use, after an earlier sample with lower levels.

We examined the question of the persistence of benzoylecgonine in the urine by studying the pattern of excretion, as measured by the EMIT test, in 35 male veterans who had recently used large amounts of cocaine and were being treated in a drug free environment. Our report confirmed the observations of Wiess and Gawin with a larger sample. There were 11(31.4%) patients who excreted benzoylecgonine for 120 hours or longer following admission. In addition 8 patients had their last positive test on the day following a negative test. The results of these studies suggest it is necessary to reassess our current understanding of the length of time that cocaine metabolites remain detectable in the urine.

The prolonged persistence of benzoylecgonine and its reappearance a! levels above 300ng/ml after a sample below this level, which was observed in this study raises a caution flag. The appearance of benzoylecgonine in the urine more than 72 hours after reported last use may no! necessarily represent repeated use and clinical judgment should be used in interpreting the meaning of such a result in order to avoid a therapeutically damaging error. Since our findings corroborate those of Weiss and Gawin, it may be important for treatment programs to reassess their current understanding of the length of time benzoylecgonine remains detectable in urine and the significance of a urine that is positive for it.

Models of recovery have been proposed both generally and for cocaine dependence specifically. For purposes of planning treatment these models can be combined to a simple time frame of early, middle, and late treatment components. Professional care is usually necessary in early and middle treatment components and may be necessary in the late component; self help groups offer considerable benefit at all stages. We believe that just as a therapeutic program needs to be tailored to match the individual's level of severity to provide an appropriate intensity of services so the urine monitoring needs to be modified to have a balance between severity and the intensity of monitoring. We suggest that an initial urine be examined to confirm the patient's history of various substances used. After the initial urine is obtained, we suggest a 5-7 day disuse period be established following which urine testing is initiated and urines are monitored daily until three consecutive negative specimens are obtained. Following this any positive urine can be considered prima facie evidence of reuse of cocaine.

In the early phase with its high relapse rate, emphasis is placed on attaining and maintaining abstinence; it is the time when closes! monitoring is necessary. Urine testing a! this time is focused on establishing when the urine no longer reflects prior use and frequent testing to detect lapses and relapses. When a drug free baseline is established random specimens can be obtained 2 or 3 days a week to monitor for reuse. Urine testing in the middle phase serves as a monitor for relapse and as a support for the patients to use as a tool as they build their own personal relapse prevention program. After sustained clean urines for 4 to 6 weeks it should be possible to decrease frequency of monitoring to 1 day week. We suggest continuing weekly random monitoring 3 to 6 months after last use then moving to twice monthly random urines for an additional 4 to 6 months. The late component is life long. Urine testing in this phase serves both the monitoring function and as a means of building an objective record of achievement. An individualized plan according to clinical picture and needs of the patient to establish an objective record of sobriety should be designed. Any further monitoring would be based on the clinical situation and a schedule mutually agreed upon by patient and therapist. In conclusion we recommend that programs monitor urine based on the severity of the clinical problem and stage of recovery starting with frequent random samples and systematically progress to less frequent samples as the clinical situation improves with concomitant increase in frequency of urine monitoring if lapses occur.

Penn-VA Center for Studies on Addiction

Desipramine Treatment of Cocaine Abuse in Methadone Maintenance Patients

I. Arndt, L. Dorozynsky, G. Woody, A. T. McLellan and C. P. O'Brien

This is the final report of a double-blind, placebo controlled trial of desipramine in the treatment of cocaine abuse in methadone maintained outpatients. After gving informed consent, 79 male veterans on methadone maintenance who met criteria for DSM-III cocaine abuse were assigned to either DMI or placebo in a 2:1 ratio. Fifty-nine patients completed the 12-week medication phase during which dosages of DMI were adjusted to give a blood level of 150-300ng/ml, if possible. An Addiction Severity Index, Beck Depression Inventory, cocaine craving scale and urine toxicology screens were done at baseline, at 4-, 8- and 12-week medication points and at the 1-, 3-and 6-month follow-up.

When the groups were compared at baseline and at the end of the twelve-week medication phase, both the placebo and DMI group improved. The improvements reached significance for the DMI group in money earned, drug use, days of reported cocaine use, days of crime illegal income, psychiatric status and cocaine craving. The placebo group also showed improvement, with lower drug use, fewer days of opiate use and fewer days of cocaine use. On ANCOVA, however, the groups differed significantly from each other in only two items, the employment pattern difference and in the days of psychiatric problems. The latter decreased in the active medication group and increased in the placebo group. The groups also showed a trend toward a difference in the days of alcohol consumption and in days working. There were no significant differences between the groups in the medical factor, days of medical problems, money earned, welfare income, drug use factor, days of opiate use, days of cocaine use, days of depressant use, alcohol factor, days intoxicated, legal factor, crime days, illegal income, psychiatric factor, Beck score, or in report of cocaine craving.

Urine toxicology results showed a high rate of cocaine and other drug use with no significant differences between DMI and placebo groups during the medication phase. Urine toxicologies are shown graphically in Figure 1.

There was no change within the groups over time or in the time vs. group interaction. The DMI group was divided into therapeutic and non-therapeutic in terms of blood level of DMI in weeks 4-12 and compared to the placebo group. There were no significant differences between the groups in drug use scores, cocaine craving or urine toxicology results.

At the 1 -month follow-up point, the placebo group had less opiate-positive urines than the DMI group, and at the 3-, and 6-month points, the DMI group had significantly more positive cocaine urines than the placebo group. See Figure 2. The DMI group also reported significantly more alcohol use than the placebo group at all follow-up points indicating a possible adverse effect.

Figure 1.
% COCAINE POSITIVE URINES IN 12 WEEK MEDICATION PHASE - DMI VS. PLACEBO

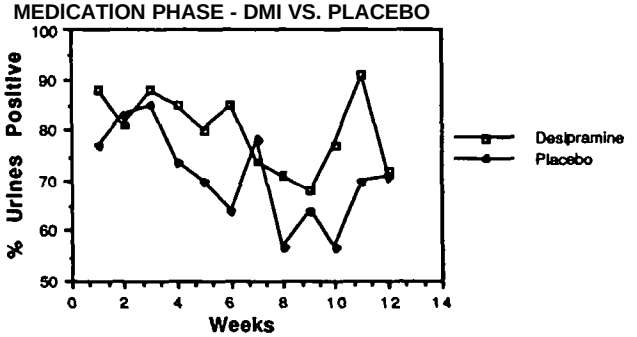
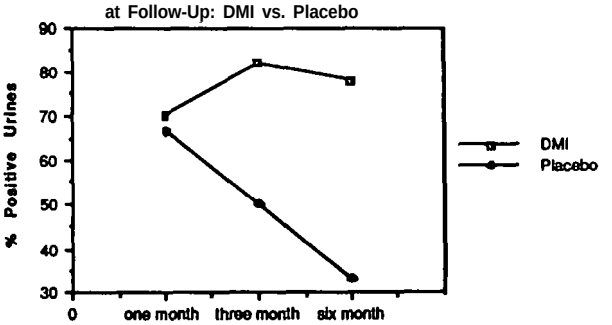


Figure 2. % Urines Positive for Cocaine at Follow-Up: DMI vs. Placebo



AFFILIATIONS: Philadelphia VA Medical Center and Univ. of Pennsylvania Medical School

This work was supported by NIDA Grant DA-03997.

A Laboratory Procedure for Evaluation of Pharmacotherapy for Cocaine Dependence

Henry Kranzler and Lance Bauer

INTRODUCTION

Childress and colleagues (1988) have found that abstinent, cocaine-dependent subjects report increased subjective desire for cocaine and exhibit increased autonomic nervous system arousal in response to cocaine-associated stimuli. These laboratory findings are consistent with those obtained in more naturalistic studies and suggest that cocaine-associated stimuli evoke responses that can precipitate relapse. The goal of our research program is to identify treatments that will reduce the magnitude of these stimulus-evoked responses and thereby reduce the probability of relapse.

METHODS

We are currently employing a laboratory procedure similar to that used by Childress and colleagues to evaluate the potential utility of bromocriptine in relapse prevention. In our procedure, patients are exposed to cocaine-associated and neutral stimuli (i.e., videotapes) in two laboratory sessions, scheduled one week apart. Subjective desire for cocaine and measures of autonomic nervous system arousal (heart rate, skin conductance level, peripheral skin temperature, respiratory sinus arrhythmia, pulse transit time) are recorded during baseline periods and during the presentation of both films. During the one-week interval between sessions, subjects receive either bromocriptine (1.25 mg b.i.d.) or placebo in double-blind fashion.

RESULTS

To date, 10 male, cocaine-dependent patients (Avg. age = 27.4 yrs., Avg. number of grams used during preceding month = 18.6) have completed the protocol. The results have thus far confirmed our hypotheses. The cocaine film evoked a greater increase in self-reported desire for cocaine than did the neutral film ($F(1, 8)=19.4, p<0.01$). This effect was larger during Laboratory Session 1 than it was during Laboratory Session 2 (Session: $F(1,8)= 6.85, p<0.04$, Film X Session: $F(1,8)=11.2, p<0.01$). The cocaine

film also evoked a greater decrease in respiratory sinus arrhythmia, an index of cardiac vagal tone, than did the neutral film, but only during Laboratory Session 1 (Film X Session: $F(1,8)=6.67$, $p<0.04$). Changes in peripheral skin temperature were not as tightly coupled to the desire for cocaine as was RSA. Skin temperature was lower during the cocaine film than it was during the neutral film ($F(1,8)=5.07, p=0.05$). However, the magnitude of this difference did not vary over laboratory sessions.

In order to relate these changes in psychophysiology to current theories of cocaine craving, we also had patients rate the intensity of symptoms of cocaine withdrawal and cocaine intoxication before and after each film. Interestingly, symptoms of acute cocaine intoxication (e.g., restlessness, heart palpitations, feeling a "rush") were rated as more intense following the cocaine film than after the neutral film. These symptoms declined in intensity over laboratory sessions and paralleled the changes in self-reported desire for cocaine and in RSA. Symptoms of cocaine withdrawal (e.g., sadness, fatigue, weakness) were not reported after the cocaine film and were not affected by any of the experimental variables.

CONCLUSIONS

These preliminary findings suggest that cocaine-associated stimuli, when presented in a controlled laboratory environment, evoke significant changes in the subjective desire for cocaine, in symptoms that are associated with acute cocaine intoxication, and in respiratory sinus arrhythmia amplitude. Subsequent analyses, conducted at the conclusion of the study, will enable us to evaluate the effects of bromocriptine on these interrelated phenomena.

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Department of Psychiatry
University of Connecticut School of Medicine

Social Impact of Crack Dealing in the Inner-City

Bruce D. Johnson, Terry Williams, Harry Sanabria and Kojo Dei

INTRODUCTION

This paper reviews the existing literature and advances a central theme: the expansion of hard drug use/abuse, and particularly the sales/ distribution of crack, is both a symptom and an important factor in the continued relative decline of inner-city communities.

METHODS

The scientific literature in several related areas were reviewed. Ethnographic research in low income communities was reviewed with particular care. Overall impression: the scientific literature can document the relative decline of inner-city communities and institutions, but generally ignores the process of decline and, especially, the impact of hard drug use and sales.

MAIN FINDINGS

The social and economic situation facing inner-city youths and young adults has deteriorated substantially since the mid-1960s in: employment opportunities, educational performance, family cohesion, and housing. In most American metropolitan areas, low income "ghettos," primarily composed of ethnic minorities, have expanded in size and become more isolated economically and socially from middle-class America.

The drug revolution has brought American society epidemics of drugs: marijuana and LSD (1960-75), heroin (1955-73), cocaine powder (1975-84), and crack (1985-present). In comparison with heroin and cocaine powder sales which are usually conducted by "free lance" sellers, crack sales are more frequently controlled by organized crack distribution groups ("crews," "posses," etc.).

Crack selling organizations can now be found in inner-cities of most metropolitan areas--These have become more cohesive. Freelance selling has been undermined by police trying to prevent "drug supermarkets." Declining wholesale prices of cocaine has increased profits for suppliers. Upper level dealers now control the work performance and gross sales by crack user-dealers and those engaged in a variety of support roles. Drug selling and the illicit drug business has expanded dramatically in economic importance: the crack economy has become a major factor in the inner-city; sales have become a major "job" for otherwise unemployable youths.

The economic wealth gained by crack distribution groups permits the self and social selection of violent persons as employees (guards), permits purchases of lethal weapons (knives, guns, semi-automatic weapons), and the systematic use of intimidation (threats of and actual violence) to control "staff," competitors, and local inner-city markets. As drug sales have become particularly prominent in the inner-city, criminality by users and dealers appear to have increased, becoming more frequent and severe.

Drug use and selling has had substantial impact on the economic wealth and subcultural lifestyles of the criminal underclass. A large percentage (usually over half) of arrestees and criminals and from inner-city backgrounds are now regular users of heroin, cocaine, and crack. Arrests for felony drug sales, especially of crack, have increased substantially since 1985. Governmental efforts to control the drug trade has led to a vast expansion (nearly doubling) of the jail, prison, probation, and parole systems since 1978.

While several persons may sell crack who are not crack users, most such sellers probably begin its use and rapidly become dependent. Most crack and hard drug abusers experience important declines in their (already low) economic well-being, and typically live well below poverty levels. Sharing needles is a primary cause of AIDS among heroin abusers: crack abusers and dealers are at high risk for AIDS via exchange of sex for crack.

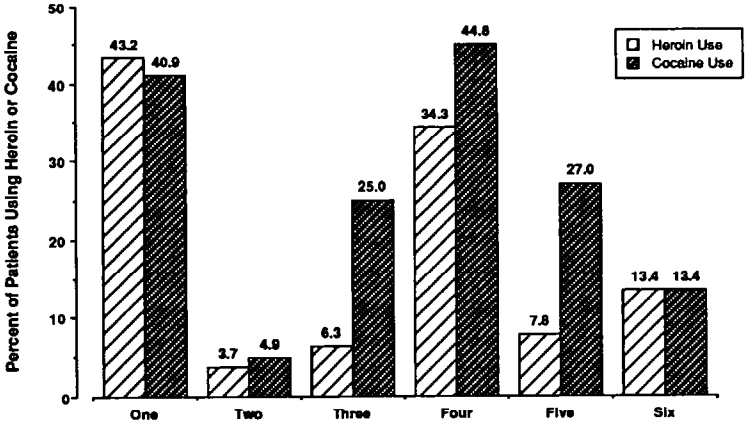
DISCUSSION

Unless important changes are made in how American society responds to inner-city problems, the already bad conditions, which are being aggravated by drug sales and abuse among substantial numbers of inner-city youths, may become even worse by the year 2000.

Affiliation: Narcotic and Drug Research, Inc.
11 Beach Street, New York, NY 10013

Cocaine and Heroin Use by Methadone Maintenance Patients

John C. Ball, Alan Ross and Jerome H. Jaffe



Although methadone maintenance treatment is effective in stopping IV drug use by most addict patients, a minority continue to use cocaine and heroin while in treatment. Factors associated with this continued drug abuse is the topic addressed in this research. The percentage of long-term patients at six methadone maintenance programs who used cocaine or heroin is shown above. Overall, 24.4 percent of the 386 male patients used heroin and 16.3 percent used cocaine in the past 30 days. But the differences in drug use by patients at the six programs were notable. Thus, heroin use was 11 times higher in one program than in another and cocaine use was 6 times higher. Analysis of these findings revealed that the factors associated with continued drug use among these patients were: inadequate programs, low methadone dosage (with respect to heroin use) and length of current treatment. For both drugs, program was the most significant factor. These findings demonstrated that some programs are markedly more effective than others.

ADDICTION RESEARCH CENTER, P.O. BOX 5180, BALTIMORE. MD 21224.

The Prevalence and Self-Reported Consequences of Cocaine Use

Alison M. Trlnkoff, Christian J. Ritter and James C. Anthony

This paper extends the initial work on U.S. cocaine prevalence which was reported on at the 1985 CPDD meeting. In the new work, prevalence estimates are based on an estimation procedure that makes the age-race-sex distribution balanced to that of the nation as a whole. Prevalence rates of cocaine use (on more than 5 occasions) were generated for certain social role and lifestyle characteristics, including educational level, marital status, living arrangements, and full-time employment status.

Data were gathered fran a sample of adults interviewed in 1980-1984 fran four sites of the NIMH Epidemiologic Catchment Area Program, including Los Angeles, (n=14,333). In addition, rates of self-reported consequences of cocaine use were calculated separately for cocaine users obtained from all five sites.

Striking differences in prevalence were found by education and marital status with lifetime rates highest among those who were cohabiting (34.5%) and lowest among those who were married (2.1%). These differences remained after controlling for age of respondent. Respondents who were employed full-time had a higher prevalence of cocaine use than those who were not. Individuals residing in households at the time of interview had a 6.4% lifetime prevalence of cocaine use, vs. hospitalized respondents (22%), and incarcerated respondents (40.3%). Cocaine use consequence rates varied greatly across sites, with self-reported tolerance (27.6%-51%) and withdrawal sickness (7.8%-33%) figuring prominently among sustained daily users.

AFFILIATIONS: Center for Nursing and Health Services Research, University of Maryland School of Nursing, 655 West Lombard street, Baltimore, MD 21201; Department of Sociology and Anthropology, Kent State University, Kent, OH 44242; Department of Mental Hygiene, Johns Hopkins School of Hygiene, 615 N. Wolfe Street, Baltimore, MD 21205.

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Characteristics of Non-Referred Cocaine Abusing Mothers

Iris E. Smith, Suzette Moss-Wells, Refilwe Moeti and Claire D. Coles

The purpose of this study was to determine whether women who had recently delivered an infant prenatally exposed to cocaine and who did- not seek treatment for their addiction could be differentiated from women who enrolled in alcohol and drug treatment programs. The experimental subjects for this study were selected from the population of women who delivered full-term infants at a major inner city hospital, who admitted to use of cocaine during pregnancy and were not seeking alcohol and/or drug treatment (n=29). The comparison group was randomly selected from the population of women who were admitted to two local drug treatment facilities during the same time period (n=50). Experimental subjects were interviewed 14 days post delivery, using the Addiction Severity Index (ASI) and the Psychiatric Symptom Checklist 90-R (SCL-90R). The same interview battery was given to the comparison group on the first day following admission to the treatment facility. All participants were required to sign an Informed Consent Form prior to participation.

Demographic characteristics, mean subscale scores on the ASI and SCL-90R, and individual item responses on the ASI were compared for the two groups of women using Analyses of Variance and Chi Square procedures. The results indicated that the non-treatment sample were less impaired in their social and psychological functioning than women who were receiving drug treatment. Significant differences were found on the Somatization ($F_{1,79}$=4.72; p<.03) and Anxiety ($F_{1,79}$=3.73; p<.05) subscales of the SCl-90R. Analyses of the ASI subscales indicated that non treatment mothers showed less impairment with regard to medical status ($F_{1,82}$=12.8; p<.001); alcohol use ($F_{1,82}$=1,73; p<.000l); drug use ($F_{1,72}$=10.8; p<.001); psychiatric status ($F_{1,79}$=8.5; p<.004); family and social relationships ($F_{1,83}$=10.2;p<.002). The women who were admitted to treatment were older ($F_{1,83}$=5.4; p<.02); better educated ($F_{1,83}$=10.7; p<.002) 'and more likely to be Caucasian. In addition, women who sought treatment were more likely to report suicidal ideation in the past 30 days and were more often rated withdrawn, hostile, anxious or distractible by the research interviewers.

Department of Psychiatry, Emory University School of Medicine

Amplitude Modulated Frequency Response During Acute Cocaine Intoxication in Rabbits

S. O'Connor, S. Kuwada, N. DePalma, T. Stanford and A. Tasman

The overall purpose of our investigation is to develop an animal model of cocaine withdrawal using EEG and evoked potential techniques. The ultimate goal is to detect electrophysiological correlates of withdrawal effects that may be transportable to studies of pharmacologic intervention in humans. An animal model is indicated because the dose and schedule of the addiction and withdrawal can be controlled. Rabbits offer several appealing characteristics as the animal of choice. Foremost is their capacity to remain motionless for long periods. This docility, even under the influence of acute cocaine intoxication, minimizes muscle movement artifact in the EEG. Lagomorph brain and blood volume are also relatively large compared to the rat. Both characteristics afford experimental flexibility when recording multi-lead EEG or correlation with repeated measure assays of serum cocaine concentration.

In humans, only scalp electrodes are practical for clinical measurements and these electrodes detect EP activity, primarily from the thalamus and cortex. Dural electrodes are preferred in the animal because their fixed location and low impedence reduces experimental variance in multi-session designs, but these electrodes readily detect neural activity from peripheral auditory nuclei. Thus, it was important to inquire whether or not cocaine has any effect below the thalamus in rabbits. The technique chosen for exploring this issue is the amplitude modulated frequency response (AMFR).

Sinusoidal modulation of a sinusoidal carrier tone excites cells specifically sensitive to the modulating frequency (MF) in all auditory nuclei. These cells emit sinusoidal responses at the MF that can be detected by Fourier Transformation of the-EEG. Incremental increases in the MF result in linear phase shifts in the transformed data from any one nucleus. The slope of the phase shift vs frequency change is a measure of conduction delay, and the amplitude at a given frequency is a measure of the intensity of neuronal activity. In addition, more peripheral nuclei have cells that are sensitive to progressively higher MFs. Thus, examination of the AMFR can identify the functional loci of the neural generators by both temporal and spectral correlations with the stimulus parameters.

Three female Dutch-belted rabbits were fitted with calvarial screws resting on the dura close to the saggital sinus at the vertex and over the frontal sinus. At the time of surgery, a teflon catheter was placed in the right external jugular and a 0.5 cm square by 10 cm long brass bar was cemented to the skull. After a week

of recovery, experiments were conducted with an ear clip serving as amplifier ground. Each rabbit was restrained by a body stocking and her head was immobilized by clamping the bar in a vice. AMFR stimuli were generated by a PDP-11/73 computer and transduced by headphones through plastic tubes firmly imbedded in customized binaural earplugs. Carrier tones were 5000. Hz at 90 dB above detected threshold. MF ranged between 10 Hz and 800 Hz with a 30 percent depth of modulation. The recorded EEG was Fourier transformed and the amplitude and phase of the peak centered at the MF was computed.

Well defined linear regions in the plot of response phase as a function of MF above 70 Hz demonstrated that the AMFR was sensitive to neural activity peripheral to the medial geniculate body. In addition, responses were evident for two structures responding to MF below 65 Hz; most probably in thalamus and cortex. Injections of saline did not change any of the results. A retrograde extension of the linear phase vs MF region below 70 Hz in response to an injection of pentobarbital supported the presumption of a thalamo-cortical origin.

Four percent cocaine HCl was diluted in saline and injections of 1 m4/Kg and 2 mg/Kg were used to test the effect of acute cocaine intoxication on the AMFR. Previous experiments had determined the seizure threshold to be at or above 6 mg/Kg. When cocaine was injected, no effect on AMFR phase or amplitude was observed for MF greater than 70 Hz, whereas dramatic changes in both the phase and amplitude properties of the AMFR were seen for MF below 65 Hz. A fourfold increase in the amplitude at MF=45 HZ was seen in response to the lower dose. The amplification occurred within 2 minutes of the cocaine injection, lasted for five minutes, and completed a monotonic decay towards baseline levels within twenty minutes.

Responses to the 2 mg/Kg dose yielded very similar results, but the amplitude in the 45 Hz range of MF was both larger and less sinusoidal since consistent harmonics in the Fourier transform were observed In response to two doses of 1 mg/Kg separated by 30 minutes, two similar transient increases were observed, but instead of returning to baseline, the AMFR amplitude continued to decay until only very small responses were observed an hour after the second dose. It is postulated that this phenomenon may be a correlate of the post intoxication “crash” experienced by humans.

This work was performed in collaboration with Yale University and was funded by NIDA, grant # D004060-02S1

AFFILIATION

Department of Psychiatry and Anatomy, University of Connecticut School of Medicine, Farmington, CT

Buprenorphine Suppresses Cocaine Self-Administration in Rhesus Monkeys

Nancy K. Mello, Jack H. Mendelson, Mark P. Bree and Scott E. Lukas

Cocaine abuse has increased among heroin dependent persons, including those on methadone maintenance treatment programs (Kosten et al., 1986, 1987; Kaul and Davidow, 1981) and has reached epidemic proportions in the general population (Kozel and Adams, 1986). At present, there is no uniformly effective pharmacotherapy for cocaine abuse (Kleber and Gawin, 1984; Gawin and Ellinwood, 1988) and the dual abuse of cocaine plus heroin is a difficult treatment challenge. The opioid mixed agonist-antagonist buprenorphine is an effective pharmacotherapy for heroin abuse (Mello and Mendelson, 1980; Mello et al., 1982) and this is the first report that buprenorphine suppresses cocaine self-administration in the rhesus monkey (Mello et al., 1989).

Five adult rhesus monkeys with a 262 ± 79 day history of cocaine self-administration were surgically implanted with a double-lumen silicon@ rubber i.v. catheter under aseptic conditions to permit treatment with buprenorphine or saline during cocaine self-administration. The i.v. catheter was protected by a custom-designed tether system that permits monkeys to move freely. An average of 64 responses was required for food (1 g banana pellet) and for i.v. cocaine (0.05 or 0.10 mg/kg/inj) on an FR4 (VR 16:S) operant schedule of reinforcement. Food sessions began at 11 a.m., 3 p.m., 7 p.m. and 7 a.m.; cocaine sessions began at 12 noon, 4 p.m., 8 p.m. and 8 a.m. Each session lasted for 1 hour or until 20 drug injections or 65 food pellets were delivered. Fresh fruit, vegetables, biscuits and multiple vitamin supplements were provided daily.

Buprenorphine was administered at two doses (0.40 and 0.70 mg/kg/day) that effectively suppressed opiate self-administration in our previous studies in the primate model (Mello et al., 1983). Daily buprenorphine treatment (or an equal volume saline control solution) was administered each day beginning at 9:30 a.m. Each dose of buprenorphine and saline was studied for 15 consecutive days (60 sessions), then buprenorphine was abruptly discontinued and daily saline treatment was resumed.

All monkeys reduced cocaine self-administration during buprenorphine treatment ($P < 0.0001$). On the first day of buprenorphine treatment (0.40 mg/kg/day), cocaine self-administration decreased by 50% or more in 4 of the 5 subjects (range 50 to 67%). Average cocaine self-administration decreased by 49% to an average dose of 1.60 ± 0.25 mg/kg/day during the first 5 days of buprenorphine treatment ($P < 0.01$). Average cocaine self-administration fell to 77% and 83%

below baseline during Days 6 to 10 and 11 to 15 of buprenorphine treatment. During the second 15 days of buprenorphine treatment at 0.70 mg/kg/day, cocaine self-administration decreased to between 91% and 97% below baseline levels. Analysis of data from individual subjects showed that both the time course and degree of buprenorphine's suppression of cocaine-maintained responding was equivalent in animals that self-administered relatively high (4 mg/kg/day) and low (2.1 mg/kg/day) doses of cocaine during the saline baseline treatment period. Individual monkeys returned to base line levels of cocaine self-administration at different rates ranging from 15 to 58 days ($\bar{x}$ 30.5 ± 10 days).

Food-maintained responding was also suppressed by 31% during the first 15 days of buprenorphine treatment (0.40 mg/kg/day). During the second 15 days of treatment with a higher dose of buprenorphine (0.70 mg/kg/day). food self-administration gradually recovered to average 20% below baseline. Although these changes in food-maintained responding were statistically significant ($P < 0.05$ to 0.01) it is unlikely that they were biologically significant. There were no correlated changes in body weight and animals continued to eat daily fruit and vegetable supplements. Analysis of the pattern of food self-administration across the four daily sessions by individual monkeys indicated that buprenorphine treatment did not change the overall distribution in comparison to saline treatment. Animals were not sedated and activity levels appeared normal. These data indicate that buprenorphine treatment suppressed cocaine-maintained responding but did not produce a generalized suppression of behavior.

These preclinical data suggest that buprenorphine may be an effective pharmacotherapy for the treatment of cocaine abuse. However, clinical evaluation of buprenorphine treatment will require double-blind (buprenorphine versus placebo) trials with randomized patient assignment and independent indices of compliance with the treatment regimen (buprenorphine blood levels) and objective measures of drug use (frequent drug urine screens). These data also suggest that buprenorphine may be potentially valuable for the treatment of dual addiction to cocaine and heroin because it suppresses heroin use by heroin addicts. Data from one open clinical trial of buprenorphine treatment are consistent with the hypothesis that buprenorphine may be efficacious for reduction of cocaine abuse, as well as heroin abuse. Opioiddependent patients treated with daily sublingual doses of buprenorphine for one month (average 3.2 mg/day; range 2 to 8 mg) had significantly fewer cocaine positive urines than patients treated with methadone (Kosten et al., 1989). If buprenorphine reduces cocaine abuse as well as dual cocaine and heroin abuse, the benefits to society in terms of reduction of drug abuse problems and the associated risks for HIV infection are incalculable. These data are in press in Science (1989). This research was supported by DA-02519, DA-04059, DA-00101, DA-00064 and DA-00115 from NIDA and RR-05484 from NIH.

Alcohol and Drug Abuse Research Center
Harvard Medical School -McLean Hospital
115 Mill Street
Belmont, MA 02178

Effect of Intravenous Infusion and Oral Self-Administration of Cocaine on Plasma and Adrenal Catecholamine Levels and Cardiovascular Parameters in the Conscious Rat

Walter R. Dixon, Andi Piang-Ling Chang, Juan Machado, Brenda Lau, Adrien Thompson, Shannon Gallagher and William Sanders

Cocaine stimulates the cardiovcascular system. The major dose-related effects are significant increases in both heart rate and blood pressure. A number of clinical studies document cardiovascular toxicity related to cocaine abuse. These symptoms include hypertensive crisis, disturbance of heart rhythm and fatal heart attacks. In this study, we have examined the effects of cocaine on cardiovascular parameters and endogenous adrenal catecholamine levels after oral and intravenous administration.

METHODS

Male Sprague-dawley rats weighing 350 grams were used in this study. Two groups of rats were used. (1) The rats were given water ad libitum and at 8:00 A.M. each morning, water was removed and the animals given their allotment of food for 1 hour followed by water or cocaine for 1 hour. The cocaine was introduced in gradual increments: 0.01%, 0.02%, 0.04%, 0.06% and 0.08% w/v on days 8, 12, 16, 31 and 39 respectively. (2) Rats were infused with cocaine (1.1 mg/kg) intravenously every 2 hours (total infusion time of 3 minutes for each infusion) for 7 days. One day before the termination of infusion and oral self-administration, the femoral artery and vein was catheterized with PE-50 tubing so that blood could be withdrawn. The blood pressure and heart rate measured at the end of 6 weeks oral administration of cocaine and after termination of cocaine infusion using a Model 7 Grass polygraph. Adrenal glands were removed after termination of cocaine infusion or-oral self-administration and homogenized in 0.4 N perchloric acid, centrifused and the supernatant filtered (nalgene syringe filter). Plasma NE, Epi and DA and endogenous NE, Epi and DA were determined by high pressure liquid chromatography with electrochemical detection (HPLC-EC). All the results were expressed as the

mean ± SE. Statistical analysis of the data was performed using Student-Neuman-Keuls test.

RESULTS

After chronic infusion of cocaine, the B.P. (99± 4mmHg) was unchanged from control (101 ± 2mmHg). However, the H.R. (308 ± 4 beats/min) decreased compared to the levels prior to cocaine infusion (328 ± 7 beats/min). After 6 weeks of oral cocaine treatment, B.P. (104 ± 3 mmHg) was unchanged from control rats (106 ± 5mmHg) not treated with cocaine. However, the H.R. decreased (270 ± 10 beats/min for oral cocaine-treated rats versus 347 ± 8 beats/min for control rats). After cocaine infusion, plasma NE levels (0.462 ± 0.032 ng/ml) were significantly higher than the NE levels (0.144 ± 0.009 ng/ml) after oral cocaine administration. Plasma Epi levels (0.959 ± 0.127 ng/ml) after cocaine infusion were also higher than the Epi levels (0.419 ± 0.155 ng/ml) after oral self-administration. The NE levels in adrenal glands (10.2 ± 1.7 ug/gland pair) after cocaine infusion is similar to the NE levels (13.6 ± 1.0 ug/gland pair) after cocaine oral self-administration. The Epi levels in adrenal glands (38 ± 1.8 ug/gland pair) after cocaine infusion is similar to the Epi (41.9 ± 4.1 ug/gland pair) levels after cocaine oral self-administration but significantly higher than the Epi levels (24.6 ± 0.8 ug/gland pair) found in adrenals from untreated control rats. Control rats infused with 0.9% saline every two hours for one week (a procedure similar to the cocaine-infusion experiment) had adrenal NE (6.4 ± 0.5 ug/gland pair) and Epi (27.0 ± 0.7 ug/gland pair) levels comparable to levels in control untreated rats. However, adrenal Epi levels (37.7 ± 1.5 ug/gland pair) of rats supplied with food on a limited basis was similar to rats on the oral cocaine regimen for 6 weeks but significantly higher than levels in control untreated rats or saline-infused control rats. These results indicate that cocaine appears to have a direct action at the adrenal medulla which may account, in part, for the increase in adrenal and plasma levels of NE and Epi. Moreover, food deprivation seems to be as effective as cocaine in increasing adrenal Epi levels suggesting that a stressful stimuli may be the initial event.

ACKNOWLEDGEMENTS

This research was supported by NIDA Grant DA-03504, and Biomedical Research Support Grant RR-5606.

AFFILIATIONS

Department of Pharmacology & Toxicology
School of Pharmacy, The University of Kansas. Lawrence; KS 66045

Cocaine Stimulates LH and Decreases PRL in Female Rhesus Monkeys

Nancy K. Mello, Jack H. Mendelson, Mark P. Bree, Maureen L. Kelly and John M. Drieze

The recent increase in cocaine abuse has been accompanied by clinical evidence that chronic cocaine use has adverse effects on reproductive function in both men and women. Hyperprolactinemia is often associated with chronic cocaine abuse and persists during cocaine abstinence (Dakis and Gold, 1985; Cocores et al., 1986; Mendelson et al., 1988, 1989). In pregnant women, cocaine use increases the risk for spontaneous abortion and may be associated with fetal malformation in the offspring (see Cregler and Mark, 1986; Smith et al., 1984; Chasnoff et al., 1989 for review). Yet, there have been surprisingly few systematic studies of cocaine's effects on reproductive hormones in humans or in animal models.

The effects of acute cocaine administration on anterior pituitary hormones were studied in female rhesus monkeys during the follicular phase of the menstrual cycle (Days 4-7). Synthetic luteinizing hormone-releasing hormone (LHRH) was used to mimic endogenous hypothalamic LHRH and stimulate release of anterior pituitary gonadotropins, LH and FSH. These studies are still in progress and this report describes data obtained under three conditions: (1) cocaine only (0.4 mg/kg. i.v.) with placebo LHRH; (2) LHRH only (100 mcg i.v.) and placebo cocaine, and (3) cocaine (0.4 mg/kg, i.v.) + LHRH (100 mcg i.v.). Ten min integrated plasma samples were collected for 40 min before intravenous administration of cocaine or equal volume vehicle control solution. After a collection of one 10 min post-cocaine plasma sample, LHRH or placebo LHRH was administered and plasma samples were collected for an additional 100 min. Samples were analyzed for LH, FSH and prolactin.

The effects of cocaine only, LHRH only, and cocaine + LHRH on LH in 3 female monkeys are shown in Figure 1. Cocaine administration was followed by a significant increase in LH ($P < .01$) of 45.8 percent. The cocaine stimulated increase in LH-was greater than that measured after administration of LHRH only (37.7 percent). Cocaine significantly enhanced LHRH stimulation of LH in comparison to LHRH and placebo cocaine conditions ($P < .01$).

FSH increased significantly within 20 to 30 min after LHRH and placebo cocaine administration. LHRH-stimulated FSH levels also were significantly higher after 0.4 mg/kg cocaine than after placebo cocaine ($P < .01$). Cocaine alone did not change FSH from baseline levels of 10.9 ± 0.4 ng/ml.

Figure 1: Cocaine and LHRH Effects on LH (N = 3)

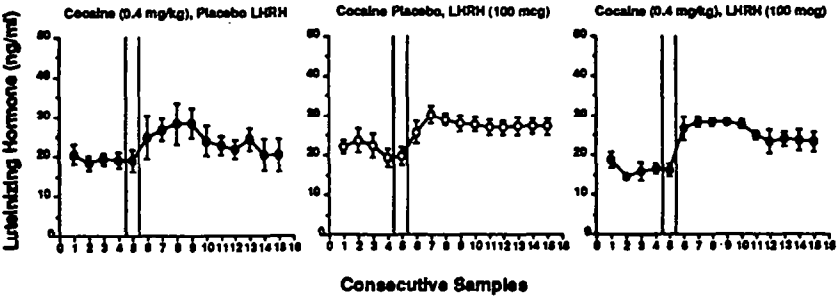


Figure 2: Cocaine and LHRH Effects on Prolactin (N=3)

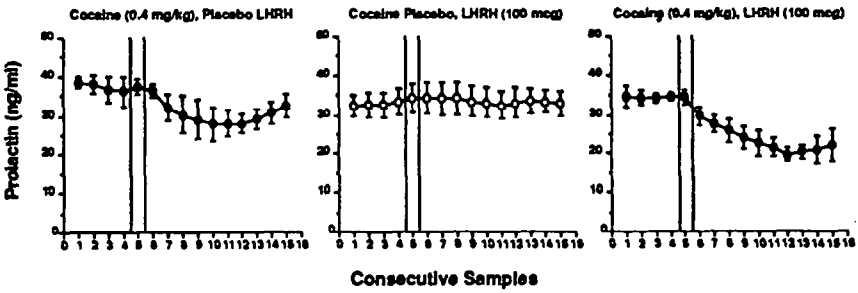


Figure 2 shows the acute effects of cocaine only, LHRH only, and cocaine + LHRH on prolactin in 3 female monkeys. Prolactin decreased significantly after administration of cocaine alone ($P < .01$) and after cocaine + LHRH ($P < .01$). LHRH had no effect on prolactin under placebo cocaine conditions. An acute cocaine-induced suppression of prolactin was also reported in male and ovariectomized rats (Ravits and Moore, 1977; Steger et al., 1981). These data are also consistent with reports that acute cocaine administration increases dopamine release in rodent (Peris and Zahniser, 1987). Inhibitory control of prolactin release is mediated by dopaminergic systems and cocaine appears to act like a dopamine agonist. An *acute* cocaine-related decrease in PRL may presage hyperprolactinemia often seen during *chronic* cocaine abuse and in abstinent cocaine abusers. This research was supported by DA-00101, DA-00064 and DA-04059 from the National Institute on Drug Abuse, ADAMHA and RR-05484 from NIH.

Alcohol and Drug Abuse Research Center
Harvard Medical School-McLean Hospital
115 Mill Street
Belmont, MA 02178

Rate Altering Effects of Magnesium on Cocaine Self-Administration

Kathleen M. Kantak, Scott I. Lawley and Stephanie J. Wasserman

Magnesium (Mg^{2+}) has been shown to have effects similar to those of stimulants such as cocaine and amphetamine. An inverted U-shape response is observed on mouse resident-intruder aggression with Mg^{2+} excesses and deficiencies. Tolerance develops to the aggression enhancing doses of MgC12, but not to the aggression inhibiting doses of MgC12. In addition, Mg^{2+} potentiates the dose dependent effects of cocaine, and attenuates the dose dependent effects of haloperidol on mouse aggression. There is also evidence that the stereotyped sniffing and locomotor stimulating properties of apomorphine and l-amphetamine are attenuated by Mg^{2+} deficiencies; and these behaviors are potentiated by Mg^{2+} excesses. In a conditioned place preference (CPP) paradigm in mice, post-conditioning injections of MgC12 were shown to potentiate cocaine induced CPP in a manner similar to amphetamine. Taken together, these data indicate that Mg^{2+}, having behavioral effects similar to other stimulants, might interact with cocaine in a rat self administration paradigm. Recent studies have shown that rats will substitute 6 mg/kg/infusion MgC12 for 0.75 mg/kg/infusion cocaine over a 10 day period. In contrast, in drug naive rats, MgC12 is not self administered. Therefore, Mg^{2+} may be a potent substitute for cocaine, but have low abuse potential. If so, then it would be expected that Mg^{2+} treatments might have rate altering effects on self administered cocaine.

Male Wistar rats were surgically implanted with a jugular catheter. All rats were infused daily with 0.5 ml of 8.5 units/ml heparinized saline followed by 0.1 ml of 1000 units/ml heparinized saline. All animals received 5 days of access to the 0.75 mg/kg/infusion training dose of cocaine before experiments began. Three experiments were performed. In the first experiment, the dose response of S.C. MgC12 on a cocaine dose response was examined in 5 groups of rats. Following 1 hr access to 2, 1, 0.5, 0.25 and 0.1 mg/kg/infusion cocaine, different doses of MgC12 (0, 15, 30, 125 or 250 mg/kg) were injected. In a second experiment, the effects of a 15% required-Mg^{2+} diet and a 100% required-control diet, fed for 3 weeks, were examined on cocaine dose responses. A third experiment examined the effects of 0, 30, and 125 mg/kg MgC12 on cocaine dose responses under both cocaine reinforcement and cocaine extinction conditions in a single group of rats.

MgC12 caused a dose dependent shift to the left in the dose response function for self administered cocaine. The reductions in responding for cocaine by MgC12 were most pronounced at the 0.1 and 0.25 doses of cocaine. In

contrast, a 15% required-Mg2+ diet caused a shift to the right in the cocaine dose response function. This increase in the rate of responding for cocaine was most evident at the 0.1 cocaine dose. The results of the first experiment were replicated by the third experiment where MgC12 reduced the rate of cocaine self administration. Furthermore, under extinction conditions, MgCl2 attenuated the normal increased rate of responding observed in the 0 mg/kg control group.

These data indicate that MgC12 reduces responding for cocaine and are therefore consistent with other data showing that MgC12 substitutes for cocaine, enhances the potency of cocaine, and has stimulant-like properties. Since MgC12 apparently has a low abuse potential, a therapeutic potential for MgC12 in cocaine abuse can be hypothesized. The reduction in extinction responding for cocaine by MgC12 may indicate that MgC12 is also capable of reducing withdrawal craving for cocaine. It is unlikely that the reductions in cocaine responding in these studies are related to motor disturbance. The response rate reductions only occurred at selective doses of cocaine; and the reductions were seen with doses of MgC12 which have previously been shown to enhance the motor activating effects of apomorphine and I-amphetamine.

Department of Psychology, Boston University, Boston, MA 02215.
Supported by NIDA grant DA04325.

Binding of [^{3}H]GBR 12935 in the Striatum, Medial Prefrontal Cortex, Nucleus Accumbens and Olfactory Tubercle of Rat

S. Izenwasser, L. L. Werling and B. M. Cox

Inhibition of dopamine uptake has been suggested as the mechanism by which cocaine produces its reinforcing effects. The striatum, while rich in dopamine terminals, is not implicated in drug reinforcement whereas the mesolimbic dopamine pathway appears to play a primary role. It is therefore possible that the binding properties and drug sensitivities of these areas might differ. Inhibition of dopamine uptake and competition against [^{3}H] GBR 12935 (1 nM) binding by GBR 12909, cocaine, amfonelic acid, nisoxetine, fluoxetine. and carbamazepine were examined in the striatum, medial prefrontal cortex, nucleus accumbens, and olfactory tubercle of rat. Additionally, competition against [^{3}H] GBR 12935 binding by methylphenidate was examined. In each of these brain regions, dopamine uptake was found to be sodium-dependent and was inhibited by GBR 12909 (IC_{50} = 5 nM), cocaine (IC_{50} = 400 nM), and amfonelic acid (IC_{50} = 1 nM). Furthermore, each of these compounds inhibited binding of [^{3}H] GBR 12935 with the following order of potency: amfonelic acid > GBR 12909 > cocaine > methylphenidate, except in the medial prefrontal cortex where amfonelic acid was much less potent. Cocaine and methylphenidate each inhibited approximately 70% of the specific binding at high concentrations, suggesting that about 30% of the [^{3}H] GBR 12935 binding was to sites insensitive to these inhibitors of dopamine uptake. Amfonelic acid recognized two sites, a high affinity site (K_I = 1 nM) and a lower affinity site (K_I = 150 nM). Competition curves for nisoxetine and fluoxetine (inhibitors of norepinephrine and serotonin uptake, respectively) showed no inhibition of [^{3}H] GBR 12935 binding at 1 µM or less and at 10 µM these drugs produced only about 40% inhibition. Furthermore, at a 100 nM concentration (well above the IC_{50}'s of 1 nM observed for inhibition of norepinephrine or serotonin uptake, respectively) nisoxetine and fluoxetine had no effects on the maximal inhibition produced by cocaine, GBR 12909 or methylphenidate, suggesting that [^{3}H] GBR 12935 was not binding to norepinephrine or serotonin uptake sites. For all of these compounds, the order of potency was the same for inhibiting dopamine uptake and competing against [^{3}H] GBR 12935 binding. Carbamazepine, however, potently inhibited dopamine uptake (IC_{50} = 1 nM) yet did not inhibit binding of [^{3}H] GBR 12935 at concentrations up to 100 µM. Thus, carbamazepine appears to inhibit dopamine uptake by a mechanism which is unrelated to the [^{3}H] GBR 12935 binding site. In conclusion, these findings suggest that the dopamine

uptake systems and their drug sensitivities are not significantly different among brain regions involved in drug reinforcement and other brain regions. Furthermore, there may be more than one mechanism by which dopamine uptake can be regulated.

Supported by a grant from the National Institute on Drug Abuse.

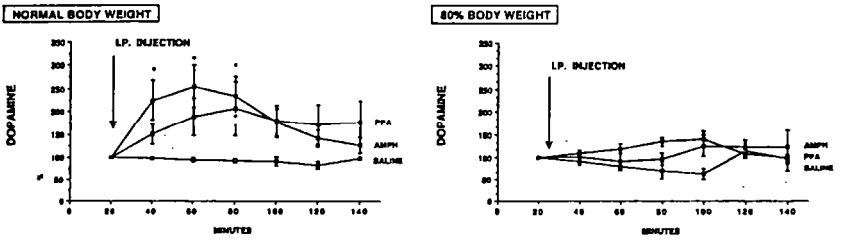
AFFILIATION
Department of Pharmacology, Uniformed Services University, Bethesda, Maryland 20814-4799.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. Animals used for this study were acquired and cared for in accordance with the guidelines published in the NIH Guide for the Care and Use of laboratory Animals (National Institutes of Health Publication No. 85-23.)

Microdialysis Studies of Psychostimulants

Bartley G. Hoebel and Luis Hemandez

In a drug discrimination paradigm, rats trained on systemic d-amphetamine (AMPH, 1 mg/kg) generalized to high doses of dl-phenylpropanolamine (PPA, 10, 20 and 40 mg/kg) in tests at 80% body weight*. Because rats will also generalize to amphetamine injected locally in the nucleus accumbens[2] and will self-inject amphetamine into the accumbens[3], we chose the accumbens for microdialysis. At normal body weight AMPH (2 mg/kg) caused significant dopamine (DA) release coupled with a decrease in DOPAC and HVA. AMPH also increased extracellular serotonin (5HT) significantly, suggesting that 5HT may be responsible for some of amphetamine's stimulus properties and behavioral effects[5]. PPA (20 mg/kg) increased extracellular DA like AMPH, which may partially explain stimulus generalization, but compared to AMPH, PPA had less effect on DA and the opposite effect on DOPAC. In rats at 80% body weight these drugs lacked their presynaptic effects on the accumbens DA system (see figure). Reducing body weight to 80% of normal weight significantly decreased basal extracellular DA in the NAC[4], while at the same time increasing basal serotonin. This suggests that changes in drug self-administration in food deprived or underweight animals may relate to changes in the mesolimbic system. Drug discrimination may also be affected by deprivation or body weight.



AMPH[5] or cocaine (COC)[6] given by way of the microdialysis probe (reverse microdialysis) increased DA and 5HT significantly and decreased DOPAC. Cocaine, procaine and lidocaine in equimolar concentrations (7.3 mM) infused by reverse microdialysis had large, small and zero effect, respectively, showing that procaine is mildly dopaminergic and that local anesthesia is not responsible for cocaine's or procaine's effect[7]. Local PCP[8] and local nicotine[8] also released DA. The effect of nicotine was blocked by i.p. mecamylamine, a nicotine antagonist. To summarize, AMPH. COC and PCP increase extracellular DA and 5HT when applied directly to the mesolimbic terminal region where amphetamine can generate stimulus properties and behavior reinforcement. Body weight is a factor in basal DA release and in the acute, presynaptic response to amphetamine.

DA released by drugs of abuse is clearly involved in positive reinforcement. particularly in the NAC where it is necessary and perhaps sufficient to reward approach and operant behavior[10]. The discovery that DA release is subject to classical conditioning[11] suggests that DA in the NAC might play a role in learning drug reward and in relapse caused by conditioned stimuli.

Semtonin release by drugs of abuse may contribute to addiction through antidepressant properties. Extracellular 5HT increased in the hypothalamus with local fluoxetine[12], an antidepressant, and amphetamine-induced release of hypothalamic 5HT increased with chronic systemic LiCl[13], both antidepressants. Inhibiting the serotonin systems with 8-OHDA eliminated conditioned suppression of saccharin intake[14]. This suggests that one function of some 5HT system is some aspect of conditioned negative reinforcement (learned avoidance). Microdialysis suggests that AMPH, COC, fluoxetine and LiCl may have 5HT effects in common, so it is possible that these drugs enable systems for escape or avoidance of aversive consequences. It is not inconceivable that serotonergic properties of some drugs of abuse could help explain addiction in terms of facilitating the escape from aversive aftereffects. In conclusion, the results support the working hypothesis that DA plays a role in drug positive reinforcement (learned approach) and suggests that serotonin plays a role in drug negative reinforcement (learned escape or avoidance). (Supported by USPHS grant DA-03597).

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AFFILIATION: Department of Psychology
Princeton University
Princeton, NJ 08544-1010

Psychostimulant Properties of MDMA

Lisa H. Gold, Mark A. Geyer and George F. Koob

The debate over the therapeutic usefulness of methylenedioxymethamphetamine (MDMA) versus its neurotoxic and abuse potentials has resulted in a renewed interest in the basic pharmacological effects of this amphetamine analog. Based on human reports of mild sympathomimetic side effects and biochemical studies indicating that MDMA activates both the serotonin and dopamine systems, the neuropharmacological basis for the psychostimulant effects of MDMA were investigated.

BEHAVIORAL PROFILE OF MDMA-INDUCED LOCOMOTION

MDMA (2.5-10.0 mg/kg, SC) dose dependently increased locomotor activity measured in photocell activity cages for at least 2 hours [1]. Characterization of the qualitative aspects of the behavioral effects produced by MDMA was conducted using the Behavior Pattern Monitor (BPM) System. Each BPM chamber consists of a black Plexiglas holeboard with three floor holes and seven wall holes, a 4 x 8 array of infrared photobeams and a rearing touch plate. A similar range of doses (1.25-10.0 mg/kg, SC) were also found to increase horizontal locomotion for at least 2 hours measured in this apparatus [2]. In addition this hyperactivity was accompanied by an initial decrease in investigatory holepokes and rearings followed by subsequent increase at the highest doses tested. Rats injected with 5.0 and 10.0 mg/kg exhibited thigmotaxis and a tendency to avoid the center of the experimental chamber, a behavioral profile similar to hallucinogen-like drugs.

Various psychostimulant drugs can produce a conditioned locomotion when tested in the presence of environmental cues that were repeatedly associated with the drug experience. MDMA (5mg/kg, SC) paired for 5 days to a distinct environment paired with the presence of an olfactory stimulus. produced enhanced locomotion during a test probe with the odor alone [3]. The observation that the stimulus properties of MDMA can also become associated with environmental cues indicates a behavioral effect which MDMA has in common with other classical psychostimulants such as amphetamine and cocaine.

NEUROCHEMICAL PROFILE OF MDMA-INDUCED LOCOMOTION

The neurochemical substrates for the psychostimulant properties of MDMA have also been investigated. The role of the mesolimbic dopamine system in the locomotor stimulation produced by MDMA was examined following 6-OHDA

lesions of the nucleus accumbens. As with amphetamine and cocaine, destruction of dopamine terminals in the region of the nucleus accumbens attenuated the MDMA-induced hyperactivity. Interestingly, rats treated with amphetamine (0.5 mg/kg, SC) after a previous injection of MDMA (5 mg/kg, SC) seem to exhibit an enhanced locomotor response or sensitization. This may reflect the behavioral effects caused by serotonin neurotoxicity associated with exposure to MDMA, as has been reported by others [4]. Thus, a loss of serotonergic inhibition of a dopamine-mediated component of locomotor hyperactivity results in an increased locomotor response. The importance of this serotonergic modulation of dopamine neurotransmission was demonstrated further by showing that a serotonin antagonist, methysergide (2.5-10.0 mg/kg, SC), markedly potentiated the increase in locomotion produced by MDMA (10.0 mg/kg, SC) [5].

CONCLUSIONS

In summary, the stimulation of locomotor activity by MDMA and the importance of mesolimbic dopamine in this response reflects similarities with the prototype stimulant, amphetamine. It is important to note that these parameters are frequently associated with rewarding aspects of drugs and drug abuse. Additionally, the behavioral profile of MDMA shares certain characteristics with hallucinogen-like agents. This mixture of stimulus properties and neurochemical actions suggests a unique pharmacology for MDMA which may be associated with potential behavioral toxicity in addition to the previously revealed neurotoxicity.

ACKNOWLEDGEMENTS

This research was supported in part by NIDA grants DA 05333 (LHG), DA 04398 (GFK) and DA 02925 (MAG).

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AFFILIATIONS

Lisa H. Gold and George F. Koob, Ph.D.
Department of Neuropharmacology
Research Institute of Scripps Clinic
La Jolla, CA 92037

Mark A. Geyer, Ph.D.
Department of Psychiatry, T-004
University of California San Diego
La Jolla, CA 92093

Comparison of the Behavioral and Neurochemical Effects of 5,7-DHT, MDMA and D, L-Fenfluramine

Stanley A. Lorens, Norio Hata, Theresa Cabrera and Margaret E. Hamilton

Three experiments were performed. In the first, separate groups (n=9-10) of male Sprague-Dawley rats (250 9) were injected with different doses of 5,7-dihydroxytryptamine (5,7-DHT; 0, 50, 100 and 150 ug/kg, i.c.v.; 30-45 min followinq nomifensine and desipramine, 15 mg/kg, i.p.) or 3,4-aethylenedioxymethamphetamine (MDMA; 0, 10, 20, and 40 mg/kg, s.c., b.i.d x 4 d) then subjected to a series of behavioral tests beginning 2-3 weeks post-injection. The rats were sacrificed 10 weeks post-injection and regional CNS monoamines and metabolites determined by HPIC. 5,7-DHT produced selective and dose-dependent but non-uniform reductions (46-95%) in regional 5-HT and 5-HIAA concentrations. The hippocampus and hypothalamus were the most and least sensitive, respectively. In contrast, MDMA produced uniform falls in CNS 5-HT and 5-HIAA levels which did not exceed 39%. The lowest dose (50 ug) of 5,7-DHT reduced open field exploration. All 5,7-DHT doses impaired swimming ability. 5,7-DHT failed to affect the acquisition of one- and two-way conditioned avoidance responses. MDMA did not affect any of the behaviors studied.

In the second experiment, rats (n=6-12/goup) were treated with 5,7-DHT (0, 50 or 200 ug, i.c.v.), MDMA (0, 10 or 40 ug/kg, s.c., b.i.d. x 4 d), or d,l-fenfluramine (FEN; 0, 5 or 20 mg/kq, s.c., b.i.d x 4 d). Open field behavior (12 min), morphine analgesia (5.0 mg/kg, s.c.), and swimming ability (14 min) were examined on the 3 days immediately preceding sacrifice either 2 or 8 weeks later. The effects of 5,7-DHT on CNS 5-HT and 5-HIAA levels were substantially greater than those produced by the substituted amphetamines. The neurochemical effects of the treatments, furthermore, were greater 2 weeks than 8 weeks post-injection. The 5,7-DHT 200 ug dose potentiated morphine analgesia 8 but not 2 weeks post-injection. No other behavioral effects were observed.

In the third, rats (n=8-12/group) were treated with either saline (1.0 ml/kg, s.c., b.i.d. x 4 d), MDMA (40 mg/kg, s.c., b.i.d. x 4 d) or FEN (12 mg/kg, s.c., b.i.d. x 4 d). The animals were food deprived and trained for 5 weeks in a 8-arm radial maze for food reinforcement. The performance of the MDMA, FEN and saline treated animals did not differ.

Repeated high doses of MDMA and FEN do not lead to dysfunctions in exploratory behavior, motor coordination or stamina, thermal pain sensitivity, morphine analgesia, avoidance conditioning, or short-term spatial memory. 5-HT neurons, furthermore, recover from the depleting effects of MDMA and FEN. The behavioral effects of 5,7-DHT appear to be test, dose and time dependent. The functional consequences of substantial reductions in CNS 5-HT levels, however, are subtle and difficult to discern.

Department of Pharmacology (Building 135), Loyola University Medical Center, Haywood, IL 60153. (Supported by NIDA Contract 271-87-8117)

Comparison of Responses by Neuropeptide Systems in Rat to the Psychotropic Drugs, Methamphetamine, Cocaine and PCP

G. R. Hanson, L. P. Midgley, L. G. Bush, M. Johnson and J. W. Gibb

Similar schizophrenia-like psychotic episodes occur following intense use of methamphetamine (METH), cocaine and pheneyclidine (PCP) and are thought to relate to the impact of these agents on CNS dopaminergic (DA) activity. Interestingly, these 3 drugs of abuse have different mechanisms of action; thus, METH induces monoamine release, cocaine blocks the reuptake of monoamines and PCP has diverse effects including blockade of the NMDA-related calcium channel as well as activation of the sigma receptor. Examination of the striatal dopaminergic system, following multiple doses (5 administrations with 6-hr intervals) of METH (15 mg/kg/dose), cocaine (30 mg/kg/dose) or PCP (15 mg/kg/dose), demonstrated that only METH significantly lowered the content of dopamine (DA) and its metabolite, DOPAC, while all 3 drugs caused significant decreases in striatal tyrosine hydroxylase activity. These DA neurochemical parameters do not seem to reflect a common mechanism for the psychotic actions of these agents.

Changes in extrapyramidal and limbic tissue content of the neuropeptides, neurotensin (NT), dynorphin A (DYN) and substance P (SP), have been shown to be indicators of fluctuations in DA postsynaptic activity. Because METH, cocaine and PCP are thought to exert their schizophrenia-like effects by altering DA activity, these peptide systems were evaluated in order to identify a common neurochemical basis for the psychotropic actions of these drugs. We observed that intense treatment (see above) with any one of the 3 drugs caused NT increases (approximately 150-250% of control) in the striatum and nucleus accumbens as well as increases in striatal DYN levels. Such common changes suggest that these peptide systems might contribute to the comparable psychotropic effects of these drugs of abuse. In contrast, the effects of these agents were distinct on the 3 peptides (i.e., NT, DYN and SP) in the nigra as well as on striatal SP and accumbens DYN systems. Perhaps these peptidergic pathways contribute to the unique pharmacological features of these agents. (Supported by USPHS grants DA 00869 and DA 04222).

Affiliation: Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Evidence of Pharmacological Tolerance to Nicotine

Heidi F. Villanueva, John R. James and John A. Rosecrans

Previous studies have demonstrated that tolerance develops to the disruptive effects of nicotine in both rats and mice in an operant response paradigm. Continuing research investigating the mechanisms of tolerance to nicotine have utilized pre- and post-session administration of nicotine to determine whether tolerance develops only to pre-session administration (behavioral tolerance) or if tolerance develops to both pre- and post-session administration (pharmacological tolerance). Preliminary investigations have demonstrated that the dose response curve is shifted to the right (indicating tolerance) following both pre-and post-session administration of nicotine. These results suggest that tolerance develops primarily due to pharmacological changes in the organism (Hendry & Rosecrans, Psychopharmacology, 77: 339-343, 1982; Meltzer, unpublished doctoral dissertation, 1980) produced by either a change in pharmacokinetic properties or by a change in receptors. However, operant studies in this laboratory and activity studies by Stolerman *et al.* (Psychopharmacolgia, 30: 329-342, 1973) have suggested that there may be a one dose tolerance effect when nicotine Is administered prior to the test session. The present studies have attempted to further separate and identify the behavioral and pharmacological factors involved in the development of tolerance to nicotine.

In the first study, 24 mice were trained to respond for sweetened milk on a fixed ratio (FR) 20 schedule of reinforcement. nice were matched according baseline responding and randomly assigned to one of three groups: saline, nicotine-l, or nicotine-14. The saline mice received 5 ml/kg immediately after each of 14 daily operant sessions. The nicotine-l mice received 1.2 mg/kg nicotine following the first operant session and 5 ml/kg saline following the next 14 operant sessions. The nicotine-14 mice received 1.2 q g/kg nicotine after each of the 14 daily operant sessions. On day 15, all mice received 1.2 q g/kg nicotine prior to the operant session. Response rates of all groups were significantly disrupted by pre-session nicotine and there was no

difference between the three groups. Post-session injections were then continued for another 14 days with the saline and nicotine-l groups receiving saline after each session and the nicotine-14 groups receiving nicotine after each session. All mice were challenged a second time with 1.2 q g/kg pre-session nicotine. The response rates of the three groups were significantly greater than during the first challenge, suggesting that all groups had developed tolerance to the disruptive effects of nicotine despite their different post-session drug histories. This suggests that there is a behavioral mechanism involved in the development of tolerance to nicotine.

Because previous studies in this laboratory had involved rats and mice trained on a variable interval (VI) schedule of reinforcement, a second study was undertaken to investigate the influence of schedule differences in the development of pharmacological and behavioral tolerance. The second study was identical to the first. with the exception that the mice were trained on a VI-15. second schedule of reinforcement. The results from the first challenge indicated that the nicotine-14 group was tolerant to the disruptive effects of nicotine, while the saline and nicotine-1 groups were not. This suggests that a pharmacological mechanism is responsible for the development- of tolerance on a VI schedule. When the mice received their second pre-session injection' of nicotine, all three groups displayed some tolerance, with the saline groups continuing to respond significantly less than the two nicotine groups. It is not possible to determine whether the development of tolerance on the second challenge is due to pharmacological or behavioral mechanisms. However, the display of tolerance in the saline group 14 days after the initial pre-session administration of nicotine does suggest that there is a one-dose effect and that this effect lasts for at least two weeks.

AFFILIATION: Department of Pharmacology & Toxicology, Medical College of Virginia- Virginia Commonwealth University, Richmond, Virginia.

Intravenous Cocaine Infusions in Humans: Dose Responsivity and Correlations of Cardiovascular vs. Subjective Effects

C. Muntaner, K. M. Kumor, C. Magoshi and J. H. Jaffe

Eight experienced IV cocaine users were intravenously. administered 0, 10, 20, and 40 mg of cocaine hydrochloride on separate days in a pseudo-randomized ascending dose series, such that the 20 mg dose always preceded the 40 mg dose. They were subsequently administered 0, 20, and 40 mg of cocaine in a fully randomized presentation order. Cardiovascular effects of cocaine were significantly different from placebo for the 20 mg but not the 10 mg dose, in contrast to subjective responses which differed from placebo for the 10 mg dose. Subjective effects of cocaine did not differ between the 20 mg and 40 mg dose conditions for the pseudo-randomized trials, but did differ in the fully randomized trials. This lack of difference in responsivity between the 20 and 40 mg dose in the earlier trials may possibly have been due to contrast effects. Cardiovascular responses were not consistently correlated with subjective responses, either within a cocaine dose condition or across doses.

AFFILIATION: NIDA, Addiction Research Center, Baltimore, MD 21224

In Utero Exposure to Cocaine and the Risk of SIDS

Barbara Lounsbury, Marta Lifshitz and Geraldine S. Wilson

The incidence of death from sudden infant death syndrome (SIDS) in infants exposed to cocaine in-utero has been reported to range from 0.55% (no increased risk) to 15% (75-150 fold increased risk). These studies raise important public health issues about the appropriate management of infants exposed to intrauterine cocaine . The wide variability in the reported incidence of SIDS in this population indicates further studies are needed.
This is a report on the incidence of SIDS in 124 infants exposed in-utero to cocaine alone or in combination with other drugs compared to 50 infants exposed only to other illicit drugs. Subjects were born in a metropolitan public maternity hospital over a one year period from 9/l/86 to 8/31/87. Mother-infant pairs were identified at the time of delivery by obstetrical and by social worker interviews. Occurrence of SIDS was ascertained by review of City of Houston death records of subjects whose cause of death was listed as SIDS, ill-defined conditions or respiratory conditions. At the time of the record review all 174 infants were at least 9 months of age. No significant differences in maternal characteristics were ‘seen except that 58% of cocaine users and 36% of other drug users were black (p=.03). There were no differentiating infant factors except significantly more frequent and severe symptoms of withdrawal in infants not exposed to cocaine (p=.OO8). No infant exposed to cocaine alone had a major congenital abnormality. Two infants exposed to cocaine in combination with narcotics had ventricular septal defect. Upon review of death records only one infant exposed to cocaine and ethanol was reported to have died from SIDS and this was confirmed by review of autopsy records. No infants in the non-cocaine group died of SIDS. This represents a risk of SIDS in infants exposed to cocaine in-utero of 0.8%. Although the-risk of this exposed group is 1.5 times the reported risk in the general population (0.54 per 1000) the 95% confidence- interval ranges from no risk to a maximum risk of 3.3%. This supports recent reports that the risk of SIDS is minimally if at all increased in ‘the in-utero cocaine exposed infants.

Baylor College of Medicine, Dept. of Pediatrics, Houston, Texas

Evaluation of Cognitive Skills in Ethanol- and Cocaine- Dependent Patients During Detoxification Using P300 Evoked Response Potentials (ERPs)

Leslie Amass, Scott E. Lukas, Roger D. Weiss and Jack Mendelson

The P300 ERP is an extremely sensitive electrophysiological measure of information processing ability believed to reflect memory updating processes, and is therefore a very useful probe for the study of cognitive functioning during protracted withdrawal. In both acute studies with ethanol (Church *et al.,* 1982; Campbell *et al.,* 1980; Lewis et *a/.,* 1970) and during acute and chronic alcoholism (Chu *et al.,* 1978; Gross *et al.,* 1988) studies have demonstrated decreased amplitude and increased latency on both auditory and visual evoked responses. Visual ERP paradigms have been highly successful in documenting depressed P300 ERP amplitudes and Increased latencies In both abstinent alcoholics (Porjesz *et al.,* 1982, 1985) and individuals at risk for alcoholism (Begleiter *et al.,* 1984, 1987; O'Connor et *al.,* 1987; Patterson *et al.,* 1987). However, several investigators have questioned the above studies (Neville *et al.,* 1985; Polich *et al.,* 1987, 1988; Steinhauer *et al.,* 1987) because of variability in both the cognitive demands of the tasks and of the subject populations, as well as the failure to replicate the findings when auditory ERP paradigms are used. At present, Information regarding the effects of chronic cocaine use on cognitive function is limited.

The recent debate as to whether P300 ERPs represent a possible biological marker for alcoholism or simply reflect the residual neurotoxic effects of ethanol exposure is currently unresolved. The present study was conducted to determine if parametric manipulations in the cognitive demands of the task are useful for discerning fundamental population differences between substance-abusing populations. Changes In information processing skills in chronic ethanol and cocaine users during detoxification were determined using P300 ERPs and reaction times generated by the performance tasks.

Sixteen non-medicated, male and female patients (22-41 yrs) admitted to a 28-day treatment program for ethanol or cocaine/ethanol dependence, and eight age-and sex-matched, non-drug using controls (21-53 yrs) provided Informed consent and were monitored once a week for four weeks. Subjects were prepared with scalp electrodes

over Cz, T_3, and T_4 sites and placed in a sound- and light-attenuated chamber for recording of EEG activity and P300 ERPs. P300 ERPs were recorded with eyes open using an *oddball* paradigm consisting of pure tone 1 kHz auditory stimuli of 100 msec duration. Subjects participated in both easy (30 dB difference) and hard (10 dB difference) auditory discrimination tasks alone and in combination with a visual reaction time task in order to measure individual responsiveness to manipulations in task difficulty and processing-load requirements.

Increased P300 latencies were evident under all hard task conditions in each group, independent of processing requirements. Reaction times, while fastest In the cocaine/ethanol group, were unaffected by manipulations in task difficulty suggesting that latency and reaction time are independent processes. Ethanol-dependent patients displayed the longest latencies and slowest reaction times overall, with P300 latency at its longest durlng hard test conditions. increased processing loads resulted in an attenuation of P300 amplitude across all groups; however, ethanol-dependent patients had the lowest amplitudes overall. P300 amplitudes showed some recovery across the four weeks in both ethanol- and cocaine/ethanol-dependent patients, although this trend was non-significant. Moreover, ethanol-dependent patients displayed markedly attenuated P300 amplitudes during easy task conditions which were strikingly similar to those seen during the hard task conditions In non-drug using controls.

These results suggest that ethanol-dependent patients' stimulus-evaluation and processing skills are more impaired than cocaine/ethanol-dependent patients during protracted withdrawal, particularly during hard task conditions. Furthermore, P300 ERPs obtained during relatively easier task conditions were particularly useful measures for delineating fundamental population differences in cognitive capacity. Further refinement of this task may yield a useful neurophysiological correlate of sensory awareness in drug-dependent populations. This research was supported by NIDA grants DA03994, DA00115 and DA00064.

AFFILIATION

Alcohol and Drug Abuse Research Center
McLean Hospital/Harvard Medical School
115 Mill st.
Belmont, MA 02178

Induction and Loss of Acute Tolerance to the Cardiac Chronotropic Effect of Cocaine in Humans

John J. Ambre, Timothy J. Conndly, Tsuen-lh Ruo and Thomas K. Henthom

Acute tolerance develops to the cardiac chronotropic and subjective effects of cocaine in humans. We have shown that tolerance to the chronotropic effect is incomplete (heart rate decline in the presence of stable plasma cocaine concentrations approaches a plateau that exceeds the baseline heart rate (Ambre et al., (Clin Pharmacol Ther 44:1-8, 1988). We also showed that tolerance development can be described as an exponential process with a half-time averaging 26 minutes. Studies described hem are aimed at full specification of a pharmacokinetic and dynamic model. Steady state cocaine infusions were used to induce a full or maximal state of tolerance. Cocaine challenge infusions were then given at intervals after full tolerance induction to determine the rate of tolerance loss.

Subjects were regular intravenous cocaine users who abstained from cocaine use for at least 24 hours prior to the study. Subjects were continuously monitored with real time display of a high resolution electrocardiographic tracing and six beat average heart rate recording. Subjects were sitting or semireclining in bed throughout. Blood samples were obtained from a forearm vein. Plasma cocaine concentrations were measured by gas chromatography/mass spectrometry. Through an intravenous line established in the opposite arm, subjects received an intravenous dose of 100 mg cocaine by constant rate injection over 10 minutes followed by an infusion designed to maintain stable plasma concentrations in the range of 1000 ng/mL. Cocaine infusions were continued for 3 hours to allow full tolerance development. A 100 mg challenge dose was administered over 10 minutes in each subject at an interval of 4 and/or 20 hours from the end of the induction infusion. Placebo studies consisted of saline infusion.

Heart rate reached a peak at the end of the 10 minute injection and then declined toward a plateau and/or declined with concentrations when the infusion is stopped The data were evaluated as plots of effect vs. concentration in temporal sequence (phase plot). Acute tolerance development was associated with clockwise opening (hysteresis) of the phase loop. Data from early (4 hr) challenge doses indicated that tolerance was still maximal. The slope of initial response was decreased and the limbs of the phase plot were superimposed (no hysteresis). Response was shown to be linearly related to concentration in the presence of tolerance (up to 1500 ng/mL). Data from challenge doses at 20 hours indicated that tolerance had been lost (or sensitivity restored). Phase plots indicated initial slopes identical to the original response and clockwise opening of the loop was evident again.

Therefore, we have shown that maximal tolerance persists at 4 hours after the induction infusion and tolerance has disappeared at 20 hours. Obviously, further studies at intervals between 4 and 20 hours will be required to fully define the rate of tolerance loss. These results, however, allow some definite conclusions about the design of a pharmacodynamic model. In our earlier studies we used .a model that allowed data description and calculation of tolerance development rate without mechanistic assumptions. Our goal is to acquired data to support a mechanistic model. Others have proposed tolerance models for other drugs. A model used for nicotine, but proposed to be generally applicable, is the "antagonist-force" model of Porchet (J Pharmacol Exp Ther 244, 231-236, 1988). A major limitation of the model is the requirement that rates of development and disappearance of tolerance be identical. With a half-time for cocaine acute tolerance development of about 30 minutes, the Porchet model predicts return of full sensitivity within 3 hours (five or six half-lives). Our results show that this is clearly not the case for cocaine (under conditions of our study, simulating typical exposure).

We propose a model of acute tolerance based on receptor pharmacology including both rapidly reversible processes that may be predominant in situations involving short term cocaine exposure and more slowly reversible processes (e.g. receptor catabolism) that are significant when drug exposure is prolonged.

SUPPORT: NIDA Grant DA04073

AFFILIATION

Departments of Medicine and Anesthesia, Northwestern University Medical School, Chicago, IL

Human Psychopharmacology of Intranasal Cocaine

Stephen T. Higgins, John R. Hughes and Warren K. Bickel

Despite the widespread use and abuse of cocaine, much remains to be learned about the human psychopharmacology of this compound. In the present study, the acute effects of intranasally administered cocaine hydrochloride (placebo, 48, & 96 mg/70 kg) on human learning and performance were investigated in 8 (5 M & 3 F) recreational cocaine users under double-blind conditions. Subjects performed the Digit Symbol Substitution Test (DSST), the Repeated Acquisition and Performance task, and visual-analog ratings of drug effect immediately before and every 15-30 min for 2 hrs after drug administration. Heart rate and blood pressure were measured every 5 min, and ECG and skin temperature were measured continuously for 1 hr predrug and at least 2 hrs postdrug administration. The 48 and 96 mg doses significantly increased overall rates of responding and the number of trials completed correctly in the DSST procedure throughout the 2 hr postdrug observation period (i.e., DSST performance was enhanced); the 2 active doses were both significantly different from placebo but not from each other. Neither overall rates of responding nor accuracy were significantly affected by cocaine in the Repeated Acquisition and Performance procedure. Visual-analog ratings of drug effect, heart rate, systolic pressure, diastolic pressure, mean arterial pressure, pressure-rate product, and skin temperature were significantly increased as an orderly function of drug dose. These effects were also generally discernible throughout the 2 hr postdrug observation period. There were no effects of cocaine on ECG. These results suggest that acute intranasal doses of cocaine do not impair learning and can significantly enhance some aspects of human performance. However, doses that enhance

performance also significantly increase cardiac output underscoring the dangers associated with cocaine use. To our knowledge, this study is the first to document that acutely administered cocaine can enhance performance in rested subjects under controlled laboratory conditions. The current widespread use of cocaine both in and outside the workplace demands that additional studies be conducted to more fully characterize that behavioral effects of this compound.

AFFILIATION

Human Behavioral Pharmacology Laboratory, Departments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401

Effects of the Combination of Cocaine and Marijuana on the Task-Elicited Physiological Response

Richard W. Foltin and Marian W. Fischman

In 1986, we reported at this meeting that combining intranasal cocaine or smoked marijuana administration with task performance increased heart rate and mean arterial pressure above levels observed following either drug or task performance alone. In this paper, we report the effects of combinations of cocaine, smoked marijuana, and task performance on heart rate and blood pressure.

METHOD

Eight adult male research volunteers, 25 to 36 years of age, with histories of cocaine and marijuana use, participated in nine experimental sessions. The study consisted of testing the effects of a single dose of intranasal cocaine hydrochloride (4, 48, 96 mg) in combination with a single smoked marijuana cigarette (1-gm, 0-2.9%Δ^9THC) administered per session. Heart rate (HR) and blood pressure (systolic, SP; diastolic,DP) were recorded every two minutes. A marijuana cigarette was smoked over a five-min period using a uniform puffing procedure. Five-min after completion of the puffing procedure, subjects were given 100 mg of white powder consisting of cocaine and lactose for inhalation. Ten-minutes of serial acquisition performance was repeated 25 and 70-min following the start of marijuana smoking. All of the subjects received low dose combinations prior to high dose combinations.

RESULTS

Cocaine alone produced dose-dependent increases in HR up to 15 bpm, and marijuana alone produced concentration-dependent increases in HR up to 27 bpm. Task performance alone increased HR by about 5 bpm. The combination of all three events resulted in peak HRs of about 37 bpm. Cocaine alone produced dose-dependent increases in SP up to 12 mmHg, while marijuana had no significant effect on SP. Task performance alone increased SP by about 7 mmHg, and the combination of task performance, cocaine and

marijuana administration increased SP to those levels seen following combinations of task performance and cocaine administration alone. A similar pattern of results was evident for DP. There was no significant effect of either cocaine or marijuana on any dimensions of performance on the serial acquisition task.

DISCUSSION

The current study pointed to the importance of measuring drug effects under conditions in which some behavioral demands are being made on the drug-taker. HR and blood pressure changes were related both to the drug administered and the concurrent behavioral requirements in the test session. Although combinations of cocaine and marijuana increased HR, these increases were no larger than those observed following the highest concentration of THC alone. In sharp contrast to the limited effect of cocaine on marijuana-induced changes in HR, is the large supplemental increase in heart rate during performance of a learning task. Combined administration of cocaine and marijuana increased blood pressure to the same degree as cocaine administration alone. As was observed with heart rate, task performance significantly incremented both SP and DP above levels seen following cocaine and marijuana administration alone. Maximal blood pressure increases observed with combinations of all three events were similar to those observed following cocaine and task performance alone.

The results suggest that HR and blood pressure elevations following illicit drug use can combine with ongoing behavior to further increase these physiological responses. Such combinations may be one factor contributing to the adverse cardiovascular consequences of drug use.

AFFILIATION

Division of Behavioral Biology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins Medical School, Baltimore, MD

Funded by DA-03476 and DA-03818 from the National Institute on Drug Abuse.

Cocaine Attenuates Opiate Withdrawal in Human and Rat

Theresa A. Kosten

Greater opiate dependence, based on the DSM-III R criteria, is associated with more severe naloxone precipitated opiate withdrawal, while greater cocaine dependence is associated with less severe opiate withdrawal (Kosten, et al, in press). This suggests that chronic or acute cocaine may reduce opiate withdrawal symptoms. To test this idea, we conducted two experiments: 1) we investigated the effects of chronic cocaine use on opiate-withdrawal in human by comparing cocaine dependent opiate addicts to non-cocaine dependent opiate addicts on measures of opiate use and withdrawal: 2) we investigated the effects of acute cocaine administration on severity of precipitated opiate withdrawal symptoms in rat.

METHOD

Chronic cocaine effects on opiate withdrawal in human. We studied 52 opiate addicts (63% male, 69% male, 33 years old) who-applied for methadone -treatment. A naloxone injection (0.8 mg/kg, s.c.) was given and ratings of opiate withdrawal symptoms were made every 5 min for 20 min. Symptoms included tearing, tremor, vomiting, yawning, restlessness, pupillary dilation, gooseflesh, and sweating. Drug dependence (DSM IIIR) was assessed with the SCID and drug use history was gathered at intake. Those subjects who met DSM IIIR criteria for cocaine dependence formed the cocaine-dependent group (n=19); those who did not formed the non-cocaine dependent group (n=33).

Acute cocaine effects on opiate withdrawal in rat. Five male, Sprague-Dawley rats (250g) were implanted with 5 morphine pellets (75 mg ea, S.C.). After 1 week, a naloxone injection (1 mg/kg, s.c.) was given and opiate withdrawal symptoms were observed every 15 min for 1 hr. Symptoms included tearing, tremor, chewing, stretch, diarrhea, ptosis, jumping,

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irritability, tail wag, piloerection, salivation, and wet dog shakes. Morhpine implants were repeated 1 week later and cocaine injections (20 mg/kg, i.p.) were given 15 min prior to the naloxone test.

RESULTS

Opiate use. Opiate use did not differ between the cocaine and non-cocaine dependent groups. Opiate dependence (total rating on SCID opiate use section) was 32.4 ± 0.2 for the former and 31.7 ±0.4 for the latter group. There were no differences in years of opiate use (12.8 ± 1.0 vs. 15.5 ± 1.9 yr) or in self rating of use frequency on a 6 point scale where 6 was multiple daily use (3.6 ± 0.2 vs. 2.2 ± 0.3).

Opiate withdrawal. Total ratings of precipitated opiate withdrawal were less with chronic cocaine use in human (15.4 ± 2.1 vs. 20.7 ± 1.8) and after acute cocaine administration in rat (16.2 ± 2.7 vs. 29.2 ± 2.1). Specifically, chronic cocaine in humans reduced tremor, pupillary dilation, and sweating; but, yawning was heightened ($p's<0.05$). Acute cocaine administration in rat reduced diarrhea, ptosis, salivation, and wet dog shakes ($p's <0.05$). There were trends for less restlessness in human and less chewing in rat with cocaine ($p's<.1$)

DISCUSSION

These results show that both chronic and acute cocaine reduce the severity of naloxone-precipitated opiate withdrawal. The data from the human study cannot be explained by differences in opiate use. Moreover, the results are consistent with the rat study, where we controlled for opiate and cocaine dose, length, of opiate exposure, and timing of cocaine administration.

Across species, the common cocaine effect on specific opiate withdrawal symptoms is the reduction in the hyperthermic response; sweating is decreased in human cocaine users and salivation is decreased after acute cocaine in rat. The mechanisms by which these cocaine effects on opiate withdrawal work are unknown.

Nonetheless, the use of cocaine by opiate addicts may be explained, in part, by their discovery that cocaine reduces opiate withdrawal severity. These results should help direct treatment strategies for this dual addiction as well as suggest new pharmacotherapies.

From the Depts. of Psychology and Psychiatry, Yale University. Supported by NIDA grant P50-DA04060. Morphine pellets supplied by NIDA.

Teen Addiction Severity Index (T-ASI): Clinical and Research Implications: A Preliminary Report

Yifrah Kaminer, Oscar Bukstein and Ralph Tarter

The Teen Addiction Severity Index (T-ASI), is a clinical interview utilizing the dimensional approach for the assessment of severity of adolescent substance abusers. The T-ASI, modeled after the ASI, is composed of seven (7) domains: substance use; school performance: family relationships; peer/social relationships; employment status; legal status and psychiatric status. The T-ASI aims to fill the need for a reliable and valid clinical instrument for youths known or suspected to use drugs. Initial psychometric studies on 50 cases indicate that this scale is both clinically useful and reliable. The clinical and research implications, particularly for improving patient-treatment matching and treatment prognosis, will be discussed.

University of Pittsburgh
School of Medicine
3811 O'Hara Street
Pittsburgh, PA 15213

Relative Abuse Liability of Benzodiazepines in Methadone Maintained Populations in Three Cities

Martin Y. Iguchi, Roland R. Griffiths, Warren K. Bickel, Len Handelsman, Anna Rose Childress and A. Thomas McLellan

The purpose of this study was to examine general patterns of licit and illicit benzodiazepine use among individuals enrolled in methadone maintenance (MM) in order to determine whether a clinical pattern of differential dependence or abuse liability might be detected. The focus of this study was on MM clients who reported experience with a variety of sedatives/tranquilizers in their lifetime.

METHODS

Subjects were selected in two phases from three (MM) clinics in three cities. In Phase I, all clients reporting to the clinics at least 3 days/week and enrolled in MM were asked to participate in a brief survey of their lifetime use of ten different benzodiazepines and barbiturates. The list of examined drugs included diazepam, lorazepam, alprazolam, clorazepate, oxazepam, chlordiazepoxide, nembutal. secobarbital, tuinal, and phenobarbital. In all, 547 clients were recruited from three clinics located in Baltimore, MD (n=50), Philadelphia, PA (n=218), and the Bronx, NY (n=279). Fewer than 4% of all subjects identified as eligible for the Phase I survey were missed or refused to be interviewed. The Baltimore clinic was 74% male, 70% white, and 30% black. The Philadelphia clinic was 100% male, 40% white, and 60% black. The Bronx clinic was 69% male, 28% white, 17% black, and 55% hispanic. Mean ages were 36, 37, and 35, respectively.

Subjects qualified for Phase II if they indicated that they had used more than seven of the ten different sedatives/tranquilizers in their lifetime. In all, 13 subjects (26%) met the Phase II eligibility criterion in Baltimore, 27 subjects (12.4%) met the criterion in Philadelphia, and 32 subjects (11.5%) met the criterion in the Bronx. In Baltimore, all 13 eligible clients were. selected for Phase II and 11 were interviewed. In Philadelphia, 15 subjects were randomly selected and interviewed. In the Bronx, 15 subjects were randomly selected and 14 were interviewed. In all, 36 white subjects, 3 black subjects, and 1 hispanic subject were recruited for the the Phase II interviews. The proportion of non-white to white participants in Phase Il was generally representative of all subjects eligible to be interviewed in Phase II.

A research assistant with no relationship to clinical services was assigned at each location to conduct the initial survey. All clients meeting the inclusionary criteria were identified and the research assistant was provided with a master list of clients to be surveyed. Clients were approached at the dispensing window and provided with the brand names and full color pictures of the ten benzodiazepines and barbiturates. Clients were asked to identify the various drugs and doses they had tried in their lifetime (licit or illicit) by circling a xeroxed representation of the various drugs. Clients were also asked to indicate if any sedative or tranquilizer use had occurred within the previous six months.

All subjects who indicated experience with from seven to ten of the drugs in Phase I were eligible for the Phase II interviews. Subjects were randomly selected and interviewed by a single investigator. The 90-minute, semi-structured interview examined each subject's lifetime prescription and non-prescription use of each drug. Chlorpromazine was also included in the interview as a negative control. Subjects were asked to provide ratings of relative preference and ratings of "high" associated with each drug. Additional questions probed reasons for use, means of acquisition, and lifetime patterns and frequencies of use/abuse. Subjects received compensation in the amount of $15.

SELECTED RESULTS AND DISCUSSION

Subjects were asked to rank order each sedative/tranquilizer with respect to relative preference. Some between clinic variation was noted, but in general the data indicated a strong and consistent preference for diazepam, lorazepam. and alprazolam over all other compounds. Subjects were also asked to rate the intensity of the "high" associated with each of the drugs on a 100mm analog scale from "not at all" to "extremely." Ratings of relative high for diazepam, lorazepam, and alprazolam. were comparable to pentobarbital and significantly greater than for oxazepam, chlordiazepoxide, and phenobarbital.

Substantial differences were also noted between the benzodiazepines with respect to proportions of subjects who reported obtaining the drug only by prescription, never by prescription, or from both licit and illicit sources in their lifetime. Fewer than 10% of all subjects reporting lifetime experience with diazepam or alprazolam stated that they used the drug only by prescription. Fewer than 18% of all subjects reporting experience with lorazepam or clorazepate reported use only by prescription. This was in sharp contrast to their reported use of chlordiazepoxide (44% by prescription only) or oxazepam (66% by prescription only).

Subjects were asked about the reasons provided to the MD regarding their need for the medication and to also provide the actual reasons for obtaining the prescription. Over two-thirds of those who obtained prescriptions for lorazepam or diazepam reported that their actual motive for obtaining the drug was, "to get high," or "to sell." Similar reports were obtained for clorazepate (50%) and alprazolam (43%). Fewer than 20% of those using chlordiazepoxide and fewer than 9% of those using oxazepam by prescription reported obtaining the prescription for reasons other than those which might be considered "therapeutic." *In summary,* the data suggest that for this population of drug abusers, there exists a clear differential abuse liability for the examined benzodiazepines. The data were consistent with respect to multiple indices of inappropriate versus appropriate patterns of use, as well as with respect to ratings of relative preference and associated "high." These findings are also consistent with previous reports from a variety of clinical and research settings which suggest that diazepam, lorazepam, and alprazolam are much more likely to be abused than oxazepam or chlordiazepoxide (Griffiths & Sannerud, 1987; Weddington & Carney, 1987; Stitzer, *et al.,* 1981; Woody, *et al.,* 1975). Great caution would appear to be indicated when prescribing benzodiazepines for addiction-prone clients.

Research supported in part by NIDA grants DA 03889 and T32 DA07209. (References available upon request).

Affiliations: University of Medicine & Dentistry of New Jersey, School of Osteopathic Medicine; Department of Psychiatry & Behavioral Sciences, Johns Hopkins/FSK Medical Center. University of Vermont College of Medicine; Bronx, VAMC; & Philadelphia VAMC/University of Pennsylvania.

Are Smokers Trying to Stop and Smokers Not Trying to Stop the Same Experimental Model?

David P. L. Sachs and Neal Benowitz

Paid, volunteer smokers, who are not necessarily trying to stop, have been reported to replace about 25% of their trough, venous serum nicotine (nic) level when using 2mg nicotine polacrilex (nic pol) and about 50% with the 4mg dose. In the present study we wanted to both refine techniques for helping subjects stay on a fixed schedule, qlh while awake, for nic pol administration and‘also to determine whether smokers trying to stop behaved in the same way as the paid volunteers used in earlier studies, who were not trying to stop smoking.

Ten subjects, highly motivated to stop smoking, were randomly assigned to receive either 2 or 4mg nic pol, double-blind, according to an every hour while awake schedule, independent of subject desire for cigarette (cig). Subjects were assessed at a cig smoking baseline session 1 week before their Target Quit Date (Visit -1), 1 day before their Target Quit Date (Visit 0), and at the end of the first week (Visit 1) and the 2nd week (Visit 2) of treatment. Subjects were referred back to their primary physician after Visit 2 for 3-6 more months of nic pol treatment. They received 30 minutes of instruction in how to correctly use nix pol at Visit 0. Venous blood was drawn in the afternoon, 1 hr after the last cig (Visits -1 and 0) or last piece of nic pol (Visits 1 and 2). At each visit vital signs and exhaled air carbon monoxide were determined; venous blood was drawn for nic and cotinine; the POMS, tobacco withdrawal symptoms, and medication (med) side effects were determined. Number of pieces of med used/day was assessed by counting the returned used and unused med as well as by concomitantly kept daily diary cards. Residual nic in used med was also assessed. Also at each visit subjects were issued daily tobacco withdrawal symptom and med side effect forms to complete each evening, using a O-4 Likert Scale. There were no differences between subjects in the 2 groups on demographic or smoking variables. Non-smoking status at Visits 1 and 2 was confirmed by exhaled air carbon monoxide $\leq$ 8 ppm.

During Baseline smoking: Se Nic (ng/ml): 18.2±10.8; 23.5±16.9(2 v 4mg p=NS); Se Cotinine (Cot)/ml): 244.2±31.4; 290.0±168.8(p=NS). At the end of the 2nd treatment week (Visit 2), results were: # Pieces/Day: 12.2±2.8; 13.2±2.0(2 v 4mg p=NS); Se Nic: 17.7±4.7;

37.8±17.8(p=NS); Trough, Venous Nic Replacement: 110±39%; 434±399% (p=NS); Se Cot: 252.6±109.6; 515.9±111.7(p<O.O2); %Nic Extracted from Used Nic Pol (measured residual nic): 75.3±7.0%; 73.0±10.5% (p=NS). Subjects using the 2mg dose obtained 1.51±0.14mg nic/piece; those receiving the 4mg dose extracted 2.92±0.42mg/piece (p<0.01).

When the 30, daily tobacco withdrawal symptom scores were averaged across the baseline smoking interval in the 2nd week of treatment, the sum of all of these symptoms showed a significant decrease for the 4mg treatment condition but not the 2mg: 0.96±O.13 (+1 SEM) to 0.77±O.21 (4mg, p=O.O3); 1.31±O.19 to 1.26±0.13 (2mg, p=NS). This total symptom score included symptoms from 3 major areas, tobacco withdrawal symptoms, med side effects, and medical symptoms, such as shortness of breath. Nearly all of the significant decrease in total symptom score in the 4mg group was secondary to decreased withdrawal symptoms, particularly self-reported craving for cigs. Each evening subjects separately assessed "craving for cigs," "desire/urge for cigs," and "physical need for cigs." Craving decreased significantly in the 4mg group from baseline smoking to the end of the 2nd treatment week: 2.63±O.43 (±SD) to 1.05±O.99, p=O.O4. During this same time period there was no change in the 2mg condition: 2.91±O.75 to 2.8O±1.08. Similarly the Total Mood Disorders Score from the Profile of Moods State (POMS) Questionnaire fell significantly in the 4mg group, 32.2 at Visit -1 to 8.0 at Visit 2 (p<O.05). Not only was there not even a decreasing trend in the total Mood Disorder Score for those in the 2mg treatment condition; rather, there was an increasing trend from 28.0 to 43.2. All 6 of the POMS subscale scores showed an improving trend for the 4mg treatment while they all worsened in the 2mg condition.

In conclusion, the 4mg dose significantly lowered total tobacco withdrawal symptom score, particularly craving for cigs; the 2mg dose did not. The 4mg dose also significantly improved the Total Mood Disorder Score on the POMS; the 2mg did not. Subjects in both the 2mg and 4mg nic pol groups maintained steady state serum nic levels during treatment that were identical to the trough, venous nic levels produced when they smoked cigs. In other words percent trough nic replacement was 100% or better in both groups. Therefore, subjects trying to stop smoking, and who were highly motivated to do so, used nic pol differently than subjects who were paid volunteers who were not trying to stop smoking. Motivated subjects extracted more nic per piece even though mean number of pieces of med used per day was similar in both groups. Consequently, paid volunteers who are not also trying to stop smoking are not necessarily the same experimental model as smokers who are trying to stop.

DAVID P.L. SACHS, M.D., Director, Palo Alto Center for Pulmonary Disease Prevention, Palo Alto, CA; NEAL L. BENOWITZ, M.D., Chief, Division of Clinical Pharmacology and Experimental Therapeutics, San Francisco General Hospital, San Francisco, CA.

This research and presentation were supported by NIDA Grants DA04986 (DPLS) and DA02277 (NLB).

Attention Problems in First Grade and Shy and Aggressive Behaviors as Antecedents to Later Heavy or Inhibited Substance Use

S. Kellam, N. Ialongo, H. Brown, J. Laudolff, A. Mirsky, B. Anthony, M. Ahearn, J. Anthony, G. Edelsohn and L. Dolan

Aggressive behavior at least as early as age six has been shown to predict heavy substance use in adolescence and probably into adulthood, particularly for males. Replicated many tims, this predictor should now be a focus of etiologic research. Less attention has been paid to shy behavior as an inhibitor of later substance use, and to attention deficits and their role int he developmental paths leading to heavy use.

We have reported earlier analyses on aggressive and shy behaviors and on the role of attention problems in predicting substance use (Kellam et al, 1983). This prior work made use of prospective epidemiological data gathered on children from first grade to age 16 to 17 from Woodlawn, a poor black urban Chicago neighborhood. We focus in this report on the baseline and evolving patterns of co-occurrence of attention problem with shy and aggressive behaviors, and with achievement scores and depression in the period of fall to spring of first grade. We use epidemiological data from children in Baltimore gathered as part of two randomized field preventive trials carried out by the NIMH Hopkins Prevention Research Center, supplemented by NIDA.

The data are teacher ratings of shy and aggressive behaviors and concentration problem gathered with the TOCA-R, a structured interview developed by Lisa Werthamer-Larsson, PhD. based on the earlier Woodlawn version: self-reports by the children of depressive symptom using a modified Kovacs Child Depression Inventory revised by Gail Edelsohn, M.D.: California Achievement Test scores, and continuous Performance Tests and other- of attentional parameters developed by A. Mirsky, PhD., B. Anthony PhD., M. Ahearn in collaboration with the NIMH Laboratory of Psychology and psychopathology.

In Baltimore in the fall of first grade, at baseline, shy children were as much at risk for attention problem as were aggressive children. Children who were both shy and

aggressive behaving (loners who fought and broke rules) were at almost twice the risk for attention problems. Attention problems were also highly related to poor achievement, and with poor achievement to depressive symptoms in children. Attention and achievement were at the center of this baseline model, with shy and aggressive behaviors on the left and depressive symptom on the right. These patterns of relationships in the fall of first grade were based on 1000 children epidemiologically sampled in the eastern half of the city in 1986. Hierarchical log linear analyses provided the basis for these inferences.

Since the data were cross-sectional, the direction of effects could not be determined, but using fall and spring data together, we could test a set of cross-lag longitudinal models to reveal evolving relationships, or directions. using only the control children, who were representative of the original total population, hierarchical regression analyses were done. The prospective longitudinal results from fall to spring of first grade reveal attention problems leading to aggressive behavior particularly among boys. In contrast, attention problem led to shy behavior among girls. Attention problems themselves in the Woodlawn data were not nearly as strong in predicting later heavy substance use as was aggressive behavior. Also in Woodlawn shy behavior predicted inhibition of later use.

The new Baltimore data suggest that aggressive behavior and shy behavior may unfold developmentally from attention problems, with important gender differences. In boys attention problems lead to the aggressive predictor, leading to increased risk of heavy substance use; in girls attention problems lead to the shy predictor, leading to reduced risk of substance use. This gender difference in the developmental paths may be related to the gender differences commonly found in the prevalence of heavy substance use in man compared to women. Attention problem appear to be an underlying condition that may lead to developmentally important behavioral, achievement, and psychopathological outcomes. Instead of a specific diagnostic entity, it may be a more general form of pathology associated developmentally with various more specific pathological expressions. The biological and enviromnental origins, as well as the developmental paths leading from attention problems should be fruitful research directions in the pursuit of etiology of substance abuse and other problem outcoms.

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AFFILIATION: John Hopkins School of Hygiene and Public Health
Department of Mental Hygiene, Baltimore, MD

The Association Between Non-Recreational Benzodiazepine Use and Other Substance Abuse

Linda B. Cottler

Dependence on benzodiazepines, or minor tranquilzers, has been referred to as the iatrogenic disorders of the '80's. Therefore, who uses them and in what patterns they are used becomes important especially if legitimate use leads to abuse. The focus of this paper is to determine whether the propensity to use other drugs and medications predicts licit use of tranquilizers. In addition, it evaluates epidemiological data for evidence of the closeness of sites of action of benzodiazepines, barbiturates and alcohol.

Data from the St. Louis NIMH Epidemiological Catchment Area survey, a study of the mental disorders in the general population, were used. Lifetime alcohol use, illicit use of benzodiazepines, sedatives, opiates, and other drugs and recent prescribed tranquilizer use were obtained on this sample of over 3000 persons. Twice as many women as men reported licit (non-recreational) use of tranquilizers in the 6 months prior to interview (13% vs. 6%) and use increased with age. In contrast, illicit use decreased with age and use rates among men were over those 3 times those of women. Prescribed tranquilizers tended to be used in combination with other psychoactive drugs. Women with a past history of a DSM-III alcohol disorder had higher rates of tranquilizer use (21%) than women with a recent history (11%) and no history (13%). These findings suggest that benzodiazepines may be successfully used to treat alcoholism or are used to substitute for alcohol. In addition, rates of prescribed tranquilizer use among persons abusing other substances were variable, but rates of illicit tranquilizer use among other substances abusers were high. When

multivariable models were used to predict tranquilizer use, use was predicted by illicit opiate use among women and illicit sedative use among men.

AFFILIATION: Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

Degree of Familial Alcoholism: Effects on Substance Use by College Males

M. E. McCaul, D. S. Svikis, J. S$_T$ Turkkan, G. E. Bigelow and C. C. Cromwell

There is convincing evidence from family, adoption and twin studies for the importance of genetic factors in the development of alcoholism in males. The present studv examined the relation of familv historv of alcoholism to the risk of other varities of drug use/abuse in male off spring. Survey data were collected from 744 male college students (age range: 18 - 25 years). who described the extent of their own drug and alcohol experience and the extent of alcohol-related problems in their first- and second-degree relatives (permitting DSM-IIIR diagnostic classification). Students' drug use data were analyzed with respect to whether they had no alcohol-dependent relatives (FHN; N=504), only second-degree (FHP-2; N=94), only first-degree (FHP-1; N=99), or both first- and second-degree alcohol-dependent relatives (FHP-1 & 2; N=43). There were no significant differences across groups for age (Mean ±S.D.= 20.6 ±1.9 years) or years of school (Mean ±S.D.= 14.2 ±1.6). The study cohort was 82% white, 14% black and 4% other.

There was a significant effect of family history status on age at first alcohol intoxication ($p<0.001$) and first use of marijuana ($p<0.001$), self-reported drinks/month ($p<0.05$), and lifetime use of marijuana ($p<0.05$). FHP- 1 & 2 students reported first intoxication at 14.1 years old, approximately one and a half years earlier than FHN or FHP-2 students. Age of FHP-1 students (14.9) also was significantly lower than FHN students. An identical pattern was found for age at first use of marijuana, which started in all groups about one year later than alcohol use. Mean drinks/month was 56.0 for FHP- 1 & 2 students as compared with 37.9 for FHN students. More than twice as many FHP- 1 & 2 students (19%) reported consumption of 100 or more drinks per month in comparison with FHN (8%) or FHP-2 (6%) students. FHP-1 & 2 students reported an average of 255 occasions of marijuana use as compared with an average of 88 occasions for FHN students.

There also was a significant effect of family history on the proportion of users (all $p<0.01$) and the number of lifetime occasions of use (all $p<0.05$) for cocaine, hallucinogens and sedatives. Generally, more FHP-1 and FIP-1 & 2 students reported any lifetime use than FHN or FHP-2 students. As shown in Fig. 1 for cocaine and sedatives, FHP-1 & 2 students reported significantly more lifetime occasions of use than students in any other group. For hallucinogens, FHP-1 students reported more use than FHN students. Finally, FHP-1 and FHP-1 & 2

students were more likely to report personal problems with both alcohol ($p<0.001$) and drugs ($p<0.001$) than FHN or FHP-2 students.

Differences in self-reported alcohol and drug use patterns and associated problems were found as a function of family history of alcoholism. The greatest level of use was found for FHP-1 & 2 students, an intermediate level for FHP-1 students, and the least in students with no affected relatives. While similar findings have been reported in offspring of alcoholic probands. this is the first report of the significant role of family history in determining the onset, amount, and broad extent of substance use in a diverse population of college males. These data highlight the need to focus future prevention efforts on these high-risk youth.

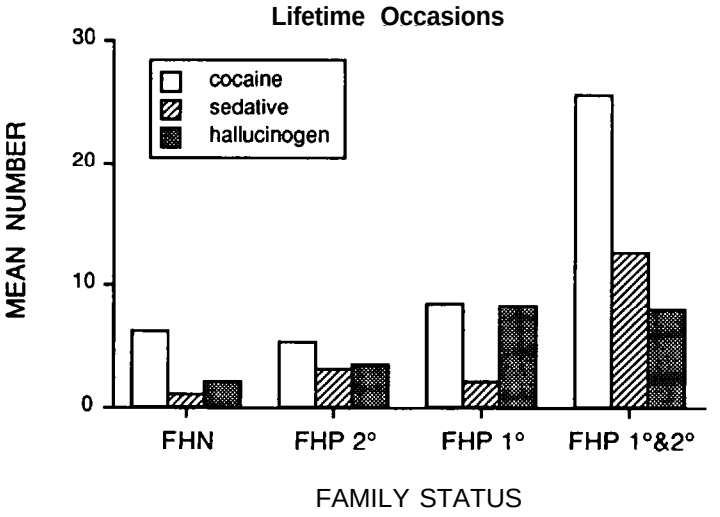

Fig.1 Mean number of lifetime occasions of use for cocaine, sedatives, and hallucinogens as a function of family history status.

AFFILIATIONS. The Johns Hopkins University School of Medicine and The Francis Scott Key Medical Center, Baltimore, MD

Preference for Ethanol in Males With or Without an Alcoholic First Degree Relative

H. de Wit and S. G. McCracken

Epidemiological evidence suggests that genetic factors play a role in the development of alcoholism. However, the mechanism by which such a biologically-based risk factor increases alcohol consumption is not known. Laboratory studies have been undertaken to determine whether individuals with a family history of alcoholism (family history positive; FI-IP) differ from those without alcoholic relatives; FHN) in terms of their physiological, subjective and behavioral responses to an acute dose of ethanol (ETH). Although differences have been reported between FHP and FHN individuals, the relationship between the observed differences and excessive alcohol consumption is not understood.

In the present study, ETH self-administration was measured directly in a laboratory study with FHP and FHN individuals, to explore the relationship between subjective responses to ETH and drinking behavior. The reinforcing and subjective effects of a low dose of ETH (0.5 g/kg) were studied in 11 male, light social drinkers with an alcoholic first-degree relative (FHP) and in 11 males with no alcoholic relatives (FHN). Candidates with a history of major psychiatric disorder, alcohol-related problems or total alcohol abstinence were not accepted. The groups were matched for age, race and drinking history. Reinforcing effects were measured using a 7-session, double-blind, double-dummy cumulative dosing preference procedure comparing ETH to a placebo. The procedure consisted of a sampling phase followed by a choice phase, during which subjects could choose their preferred dose of ETH (or placebo). Subjective effects were assessed before and after ingestion of beverages, using the Profile of Mood States and a visual analog

scale.

The FHP and FHN groups both chose ETH on about 60% of choice sessions, and they ingested average doses of 0.9 g/kg. The groups did not differ in ratings of drug liking, identification of the ETH and placebo beverages, psychomotor impairment after ETH or breathalyzer ETH levels. On the POMS, the groups did not differ in their responses to ETH, but the FHP group scored marginally higher than the FHN group on several scales indicative of dysphoric mood (e.g., higher on Fatigue, lower on Friendliness) independently of drug administration. The only difference between the groups in response to ETH was on the visual analog scale, where the FHP group reported a slightly faster onset of ETH effects ("feel drug" and "high" scales).

These findings suggest that a family history of alcoholism does not necessarily confer on other family members a differential subjective response to ETH, or an increased tendency to consume the drug. However, these conclusions may be limited by the sample of individuals accepted into the study: The participants were a highly functioning, university-based sample, who were carefully screened for any history of psychiatric and drug-related problems. Differences in responses to ETH between FHP and FHN individuals may only emerge in a less highly selected sample.

This research was supported by USPHS grants DA02812 and M01RR00055.

Affiliation: Department of Psychiatry, Pritzker School of Medicine, The University of Chicago, Chicago IL 60637

Sedative/Tranquilizer Use and Abuse in Alcoholics Currently in Outpatient Treatment: Incidence, Pattern and Preference

Barbara Wolf, Martin Y. Iguchi and Roland R. Griffiths

Sedative use plays a significant, although often underestimated role in alcoholics; there are reports that 30-50% of the patients admitted for alcohol treatment are currently using sedatives (Busto et al: Br J Addict 78 : 429-435, 1983; Sokolow et al: Br J Addict 76 : 147-158, 1982). Moreover, there is an ongoing controversy with respect to the therapeutic use of benzodiazepines in patients with alcohol abuse or dependence. While some clinicians argue that benzodiazepines are strictly contraindicated in alcoholics because of their increased risk to abuse these compounds, other clinicians claim that they may be used safely in the majority of alcoholics, when taken under close medical supervision and for relatively short periods of time.

AIMS: The purpose of this study was to examine the life-tune exposure, incidence of extended intake, pattern, dosage. and duration of sedative use in alcoholics who were in outpatient treatment. Additional questions addressed the sources of the drugs, reasons for their use, their therapeutic, recreational, and adverse effects. Another focus was on drug preference and differences between various compounds with respect to the reported euphoriant effect.

METHOD: The data are derived from an interview study which was conducted during a 10 week period and was aimed to include all patients currently enrolled in **an outpatient** treatment center for alcoholics. The study consisted of two parts, a screening interview and a detailed interview. During the initial interview all patients were asked, if they ever had used sedatives, and, if so, which drugs and for how long. Those patients who reported a life-time experience with sedatives exceeding 30 days were asked to participate in a paid structured detailed interview.

RESULTS: The total target population for the initial interview consisted of 139 patients; 118 or 85% of the patients completed the initial interview. Of these 118 patients. 29% reported having no experience with sedatives, 25% having a limited experience of less than 30 days, and 46% having a life-time exposure exceeding 30 days. An evaluable detailed interview was obtained in 46 clients who represent the designated “experienced” patients; there was a statistically significant preponderance of females in this group. The majority of the “experienced” sedative users were in fact polydrug abusers, a high proportion of them having experience with other drugs such as marijuana, stimulants, and cocaine. Sixteen patients or 35% reported continued sedative use during the current outpatient

treatment; only four of them received them by prescription, the rest from illicit sources. The patients reported having used of a variety of sedatives recently, predominately benzodiazepines (BZD). Diazepam (Valium) was by far the most frequently mentioned sedative (89%), followed by methaqualone (Quaalude) and lorazepam (Ativan). The overall duration of use of any kind of sedative was longer than one year in 81% and exceeded 5 years in 46%. Sixty-seven percent of the patients reported having used one or more sedatives daily for some period of time. The BZD were considerably more frequently used on a daily basis than the non-BZD; this, however, may reflect more the difference in availability than in drug preference. Seventy percent reported using doses higher than therapeutic BZD doses (30mg diazepam or an equivalent dose of another BZD derivative). in 33% even exceeding 80mg diazepam. In 61%. the sedative use could be classified as abuse; this included higher than therapeutic doses, not for therapeutic purposes, but to get "high" and/or counteract the effects of other drugs, combination with alcohol, and illicit sources. Fifteen percent reported an appropriate use of BZD as medically prescribed; none of these seven patients had a history of polydrug abuse. Twenty-four percent reported both use and abuse of different compounds or using the some compound in different ways at different times. Initially, the primary reason for sedative use was to get get "high" in the majority of patients. However, the motive for using sedatives changed over time in many of them. The proportion of patients taking sedatives to treat alcohol withdrawal symptoms, to counteract the adverse effects of other drugs, primarily cocaine, and to relief symptoms such as anxiety, sleeping difficulties, and tension considerably increased over time. The non-BZD were obtained almost exclusively from illicit sources. Within the BZD class, diazepam showed the highest proportion of "street" use, while clorazepate (Tranxene) and flurazepam (Dalmane) were obtained mainly by prescription. The patients were asked to rate the preference and the intensity of the "high" (100mm analog scale) of each sedative with which they had experience. These two measures covaried. Ratings of drug preference and intensity of the "high" showed that diazepam was not different from methaqualone, ethchlorvynol (Placidyl), seco/amobarbital (Tuinal), lorazepam or alprazolam (Xanax), but was significantly greater than chlordiazepoxide (Librium) and clorazepate (paired t-test comparing pairs of drugs in subjects with experience with diazepam and the comparison drug).

CONCLUSION: About one half of the surveyed alcoholic patients had a history of past or current sedative use. Only a relatively small proportion of the patients. in particular those without concomitant polydrug abuse. used the sedatives/tranquilizers appropriately, while the majority abused some or all compounds. With respect to the intensity of the "high" and drug preference, diazepam was rated similarly to lorazepam and alprazolam, as it also was to methaqualone. ethchlorvynol. and Tuinal . A difference within the BZD class with respect to the abuse liability was suggested by preference and intensity of the "high" which were rated greater for diazepam. lorazepam, and alprazolam than other compounds such as chlordiazepoxide and clorazepate. These differences may have an impact on the prescribing practices of BZD to alcoholics.

Supported by NIDA grant DA-03889

AFFILIATIONS: [1]The Johns Hopkins University, Francis Scott Key Medical Center, Baltimore, MD; [2]University of Medicine & Dentistry of New Jersey, Camden, NJ

A Tale of Three Cities: Risk Taking Among Intravenous Drug Users

Robert Booth, J. T. Brewster, Stephen Koester, W. Wayne Wiebel and Rick Fritz

In the United States, 20% of the 98,000 diagnosed AIDS cases as of June, 1989 were ibtravenous drug users (IVDU), and another 7% gay or bisexual IVDUs. Among intravenous drug users AIDS appears to be transmitted by small amounts of blood contained in shared needles, syringes, or "cookers". Studies have found a direct relationship between the frequency of injection, the number of partners shared with, the amount of needle sharing, and HIV seropoeitivity. Research has also shown a relationship between the number of sexual partners of IVDUs and seropositivity.

The present investigation was initiated to add further understanding about risk-taking behaviors among intravenous drug users. A total of 345 IVDUs were interviewed from Baltimore, El Paso and Denver. Subjects were screened for sighs of recent intravenous drug use prior to inclusion in the study. Their average age was 35 years, with 58% between 30 and 40. Males constituted 69% of the sample. Ethnically, 50% were black, 31% Hispanic, and 15% white. Self-report data revealed 86% had injected cocaine in the six months preceding their interview, 83% heroin and 68% speedballs (combined heroin and cocaine). The average age of first injection was 20.3 (range 10-56) and weekly injection 22.9 (range 12-77).

Assessed risk behaviors included frequency of injection, sharing needles and "cookers", number of needle partners, and number of sexual partners. Protective measures associated with these activities consisted of cleaning needles with bleach or other disinfectants, as well as use on condoms. Over 50% reported injecting drugs daily and 70% shared needles with others, averaging 6.3 partners in the past six months. In addition, 86% had shared a "cooker" and nearly 50% injected in a "shooting gallery". More than half of the males sampled had two or more sex partners, including 18% with five or more. Females averaged 19 sex partners in the preceding six months with 22% reporting sex with five or more. Two-thirds of the total sample never used a condom, while only 6% always used this form of protection.

On the other end of this risk continuum are those IVDUs who took precautions to avoid AIDS. These included 29% who did not share needles, 24% who always cleaned their needles with an effective agent, 8% who had no sexual relations and 6% who always used a condom. Subjects following such practices could be considered low risk if they adopted safe behaviors in other associated areas of their lives. However, in an analysis of total risk, it was found that only 14 subjects (4%) practiced safe needle use (including sharing a "cooker") and safe sex.

Despite these findings, forecasting a g-rim future for intravenous dnlg users, some encouraging results were seen. Of the 14 low risk subjects, 9 were from Baltimore, nearly two-times their sample representation. Even more telling was an analysis of risk according to location: Baltimore subjects were significantly less at risk according to number of needle sharing partners, borrowing needles, sharing a "cooker", injection in a shooting gallery, cleaning needles, use of disinfectants, number of sexual partners and use of condoms than either their cohorts in El Paso or Denver.

Street outreach to modify risk behaviors among IVDUs began in Baltimore approximately two years prior to funding in El Paso and Denver. These results appear to suggest that there may be a potential to moderate risk through intervention.

AFFILIATION:

UNIVERSITY OF COLORADO HEALTH SCIENCES CENTER
DEPARTMENT OF PSYCHIATRY
ADDICTION, RESEARCH AND TREATMENT SERVICES
DENVER, CO

UNIVERSITY OF ILLINOIS SCHOOL OF PUBLIC HEALTH
URBANA, IL

Supported by the National Institute of Drug Abuse

Diagnostic Agreement Between DSM-III and DSM-III-R Dependence Disorders

Linda B. Cottler and John E. Helzer

The DSM-III-R criteria have recently been published and include major revisions in the concept of psychoactive substance dependence. For example, in DSM-III-R, tolerance and withdrawal are among 9 symptoms which have equal weight, so neither is necessary for a diagnosis. Three out of 9 symptoms are required for a dependence disorder: abuse is a residual category. A duration criterion is necessary, yet is vague and subject to interpretation. Many factors might affect the diagnostic agreement between 2 systems. Reliability of the items used, clarity of questions number of questions for each criterion, and the extent to which questions accurately reflect criteria will have an impact.

A reliability study of the CIDI Substance Abuse Module, an interview assessing both DSM-III and III-R criteria, offered us the opportunity to evaluate diagnostic agreement between DSM-III and III-R dependence criteria. Our sample included 39 persons in treatment for substance abuse or dependence. The rate of system overlap, percent agreement and percent positive in both systems, and Kappa values are reported to evaluate whether one system is more stringent than the other. Alcohol and marijuana dependence were more likely in the DSM-III-R system. Only half of the persons diagnosed with DSM-III-R cannabis dependence were also diagnosed as dependent in the DSM-III system. Tobacco dependence was more likely to be diagnosed in the DSM-III system. Diagnostic agreements were highest for amphetamine, opiate and sedative dependence.

Reasons for differences and overlaps are discussed, as well as the clinical and epidemilogical implications of this preliminary work. Further studies are underway using a more heterogeneous population.

AFFILIATIONS: Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

In Their Own Words: Drugs and Dependency on New York City's Streets

Alisse Waterston

This paper examines problems of drug dependence from the perspective of users themselves whose involvements and experiences in the work force, the shelter system, the criminal justice system, and personal networks are presented. My approach to understanding street addicts. differs from the most widely accepted conceptualization by social scientists, policy makers, and perhaps, the general American public. In this "traditional" conceptualization, street addicts and addict "subculture(s)" are peripheral, marginal and deviant.

A brief look at the intellectual history of the drug use field provides some context for my conceptual alternatives. Social science interest in drug users emerged from the topical and methodological specialties of the Chicago school of urban studies. Social science attention on urbanism highlighted the seamy side of life, urban decay and disorganization, with a particular focus on minorities, gangs, opium addicts, hoboes and juvenile delinquents. Each study seemed to contain a world unto its own, while also representing inevitable, undesirable features of city life. Studies of social problems led to the development of social deviance as a sub-specialty, in which drug use was a substantive area of study. This approach directs attention to the deviant subculture, and remedies are directed at deviant actors. The fit between studies in social deviance and culture-bound field studies rests on a common outcome of their approaches: distancing and exoticizing the subject.

In my view, a more accurate perspective addresses the systematic interrelationship between street addicts and the larger political, economic, social and cultural systems within which they live. Based on two ethnographic studies of street addicts in New York City, I have attempted to wed micro-phenomena -- data on daily of street addicts -- to macro constructs and

explanations. The experiences of street addicts suggest that their lives are constantly and continuously shaped by relations within a range of "public" and "private" arenas. The focus *here* has been on understanding their experiences in terms of larger constraints, inequality, conflict, institutionalized social control, dependency, accommodation, and the role of dominant cultural values. Today's "drug scene" is best understood as a feature of late capitalism in which addicts serve particular economic, political and ideological functions. Briefly, the economic functions of street addicts center on their position as low-wage, vulnerable and disorganized workers, as bearers of costs in their daily reproduction, and as consumers of goods produced by the international drug trade. The most important ideological function of street addicts and the symbolic images of deviance and decadence they provoke, is in providing a convenient scapegoat for society's ills. Frequently drawn from the city's poorer neighborhoods which are largely inhabited by racial and ethnic minorities, drug users are easily blamed for their individual bad habits and the destructive outcomes of drug use. As a social group, street addicts also serve a political function. In their present form, street addicts are a captive entity, separated form their class by mandates and practices in the state management of "deviants." Fashioned into a separate group and ideologically separated, street addicts function as a divisive tool within their class, ensuring that conflict not spill outside the boundaries of poor and working class communities.

The findings presented here unmask some long-held as sumptions about drug users, and suggest a rethinking of social program goals and approaches. The consequences of our current social responses to the "drug problem" must be examined since many of the difficulties faced by street addicts stem from the policies and practices we have helped shape. It is our responsibility, therefore, to step back and honestly reflect upon our contributions to the problems of drug dependence.

Narcotic and Drug Research, Inc.
11 Beach Street
New York, N.Y. 10013
and
Department of Anthropology
City University of New York
Graduate Center 33 West 42nd St.
New York, N.Y. 10036

Outpatient Maintenance/Detoxification Comparison of Methadone and Buprenorphine

Rolley E. Johnson, Paul J. Fudala, Charles C. Collins, Jerome H. Jaffe

A 180-day, phase II clinical study is being conducted in 150 outpatient volunteer subjects to compare the effectiveness of buprenorphine or methadone in treating opiate dependence.

Subjects are males and females between the ages of 21 and 50 years. Criteria for admission include 2 opiate-positive urine samples (with no more than 1 methadone-positive sample) and a history consistent with opiate addiction. Subjects are required not to have participated in a structured methadone or buprenorphine maintenance or detoxification program for more than 7 total days within 12 months of beginning the present study. Other exclusionary criteria include pregnancy, a psychiatric severity score of 7 or greater on the Addiction Severity Index, and active cardiovascular or hepatic disease. While potential subjects may be polydrug users, those who are found to have a dual dependency as assessed by clinical interview are excluded from the study.

Subjects are stratified into drug and dosage groups according to sex, age, and response to a naloxone challenge injection (0.4 mq IM) using the Clinical Institute Narcotic Assessment score. Medication doses are given double-blind, double dummy (i.e., methadone orally, buprenorphine sublingually). Subjects are monitored by medical staff for adverse drug effects. Those who cannot tolerate or be maintained on their prescribed dosages are provided a 21-day methadone detoxification. Subjects are required to participate in individual counseling sessions (30 to 60 min per week) using a relapse-prevention model. Voluntary group sessions are also offered.

The primary outcome measure to assess treatment effectiveness will be the number of urine specimens positive for illicit drugs using a weighted analysis based on time in treatment. Secondary outcome measures will include retention time in study, number of missed clinic visits, subject-reported side effects and depression, subject-reported opiate-like and withdrawal effects, and results from blood chemistries and urinalyses.

AFFILIATION: NIDA, Addiction Research Center, Baltimore, MD.

Time Course of Repeated Naloxone Challenge After Single Morphine Doses in Humans

Stephen J. Heishman and Maxine L. Stitzer

Acute physical dependence refers to the abstinence syndrome precipitated by an opioid antagonist following a single dose of an opioid agonist (Martin & Eades, 1964). Previous studies in our laboratory have demonstrated the occurrence of naloxone-precipitated abstinence from 45 minutes (Heishman & Stitzer, 1988) to 6 hours (Bickel *et al.,* 1988; Heishman *et al.,* 1989) after acute morphine administration in nondependent humans. The purpose of this study was to determine whether naloxone-precipitated abstinence could be observed as long as 24 hours after a single morphine dose and whether the abstinence syndrome could be repeatedly elicited by two successive naloxone challenges.

METHOD

Participants were six males 24-37 years old, reporting a history of prior opioid use of 2-18 years and average current opioid use of 8 times per month. Subjects lived on an inpatient research ward during the 5-week study. No abstinence symptoms or signs were observed in any subject after a 10 mg/70 kg naloxone challenge before the start of the study. Subjects participated in eight experimental sessions in which they received i.m. injections of morphine (18 mg/70 kg) followed 6 and 24 hours later by i.m. naloxone (10 mg/70 kg) or placebo challenge. Subjects were exposed twice to each of four experimental conditions: placebo (6 hr)-placebo (24 hr), placebo (6 hr)-naloxone (24 hr), naloxone (6 hr)-placebo (24 hr), and naloxone (6 hr)-naloxone (24 hr). Experimental sessions involved baseline measurements before the naloxone or placebo challenge, followed by a 60-min post-challenge assessment period- Physiological measures were recorded continuously throughout sessions and a battery of pupil photographs and subjective and observer ratings of abstinence was completed at baseline and 5, 15, 30, 45, and 60 min post-challenge.

RESULTS

Naloxone challenge at 6 hr postmorphine reversed morphine-induced miosis. respiratory depression, and subjective reports of opiate symptoms, drug high, good drug effects, and drug liking. At 24 hr postmorphine, naloxone had no effect on these agonist measures, which had returned to premorphine levels and were no longer measureable. However, at 6 or 24 hr postmorphine. naloxone precipitated subjective reports of withdrawal symptoms, bad drug effects, and withdrawal sickness. Additionally, at both times, naloxone significantly increased observer ratings of Himmelsbach abstinence signs, primarily yawning and perspiration Subjective ratings of bad drug effects and withdrawal sickness were significantly less at 24 hr than that at 6 hr postmorphine. However, composite withdrawal

symptoms and observer-rated abstinence signs did not differ in intensity at 6 and 24 hr postmorphine.

When naloxone challenge at 24 hr was preceded by naloxone at 6 hr postmorphine, the magnitude of abstinence symptoms and signs was clearly attenuated- Subjective ratings of withdrawal symptoms, bad drug effects, withdrawal sickness and observer-rated abstinence signs were slightly elevated, but not significantly greater than respective placebo levels.

DISCUSSION

This study demonstrated that a naloxone-precipitated abstinence syndrome can occur at least as long as 24 hr after a single dose of morphine in nondependent humans, when agonist effects are no longer observable. This suggests that morphine induces some biological change persisting beyond its measureable effects, which may represent the beginnings of opioid physical dependence. These results also indicated that when naloxone challenge at 24 hr was preceded by naloxone at 6 hr postmorphine, the precipitated abstinence syndrome at 24 hr was attenuated, at least with these drugs. doses, and temporal interval. This is consistent with the hypothesis that initial naloxone administration reset receptor mechanisms for the development of opioid physical dependence (cf., Krystal *et al.,* 1989). However, it is possible that with a longer acting agonist, larger doses of agonist or antagonist, or a shorter interval between the first and second antagonist challenge, one might observe repeated antagonist-precipitated abstinence following acute agonist treatment.

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ACKNOWLEDGEMENTS

This research was supported by USPHS research grant DA-04011 and research training grant DA-07209 from the National Institute on Drug Abuse.

AFFILIATIONS

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine (SJH and MLS) and Addiction Research Center, National Institute on Drug Abuse (SJH). Baltimore, MD 21224

Evaluation of the Abuse Potential of Picenadol

Donald R. Jasinski and Kenzie L. Preston

The agonist/antagonist analgesic picenadol is a racemic mixture of an opioid agonist (d-isomer) and an opioid antagonist a-isomer). Picenadol also has anticholinergic activity. Two studies were done to assess its abuse potential In the first, picenadol 18.75, 37.5, and 75 mg, morphine 15 and 30 mg, and placebo were given IM every other day under double blind conditions to 6 adult male opioid abusers according to a latin square design. Structured questionnaires administered at fixed time intervals before and after dosing measured drug identification, degree of subjective change, euphoria, dysphoria, sedation and symptoms.

Picenadol 75 mg was distinguished from morphine by sedation, dysphoria, and hallucinatory activity, probably from anticholinergic activity. Picenadol 18.75 mg was morphine-like. Picenadol 37.5 mg was morphine-like with some dysphoric effects. The second study was similar except for dosages. Picenadol 12.5, 25, and 50 mg were compared with placebo and morphine 10 and 20 mg. At these doses, picenadol produced morphine-like effects and had some dysphoric effects but was not hallucinogenic.

Picenadol had less euphorigenic potential than morphine and, in our opinion, has a lesser abuse potential.

AFFILIATION

Francis Scott Key Medical Center, Johns Hopkins University School of Medicine, Baltimore, MD.

A Pilot Study of a Neuro-Stimulator Device vs. Methadone-in Alleviating Opiate Withdrawal Symptoms

Elsayed Elmoghazy, Bruce D. Johnson and Frederick A. Alling

INTRODUCTION

Methadone with gradual withdrawal is the only FDA-approved technique for detoxifying from heroin or opiate dependency. Cerebral electro stimulation appears to be a promising nonchemical alternative for detoxification from opiates. but the results of prior research is mixed, and the biochemical mechanism of action is not documented.

RESEARCH QUESTION

Can a Neuro Stimulator Device (NSD) be as or more effective than methadone in overaIl results and in alleviating a variety of opiate withdrawal symptoms?

METHODS

The Neuro Stimulator Device (NSD) is an experimental proprietary device, similar to a walkman, which weighs about a half pound, generates a weak current with frequency range of 50-300 HZ and pulse duration of 30 MSEC. Two wires lead to surface electrodes taped over the mastoid bone behind each earlobe. A pilot studv of the NSD involved 125 patients at St. Luke's-Roosevelt Hospital (Manhattan) for 7-day opiate detoxificaton between 11/87-4/88. All addicts applying for admission were invited to participate. Patients who gave informed consent wore the NSD, but were randomly assigned and blinded to two experimental groups. Patients in the "NSD" group received the active NSD and placebo methadone; those in the "methadone" group received regular declining doses of methadone but a placebo wave in the NSD device.

The pilot study encountered usual problems of research with addicts. Less than half of the eligible agreed to participate; the dropout rate was over half among those in both groups. Efforts were made to double blind the monitors, but these were not always successful. Electrodes frequently came off, batteries on the NSD failed. Clear records of whether and how long subjects wore the NSD were not systematically maintained. Data collection procedures changed during the study. Thus, the results reported below are very preliminary and based upon less than adequate methods.

FINDINGS

The Medical Director rated all patients on presence of objective withdrawal symptoms from none, to low-moderate and high during their stay in the locked detoxification ward. The results show that patients in the NSD group did, at least, as well as those in the Methadone group.

Table 1. Patterns of Objective Withdrawal Symptoms During Dextoxification Among Experimental Subjects

Patterns of Withdrawal Symptoms Over Time	Experimental Groups: NSD Active NSD Placebo Methadone		Methadone Placebo NSD Wave Declining Doses Real Methadone		
	N	%	N	%	T-Test
No Withdrawal Symptoms	7	18	7	24	
Objective Symptoms Reduced	5	13	3	10	NS
Mixed Objective Symptoms	27	69	19	66	NS
Total	39	69	29	100	NS

In 3-4/88. 17 subjects responded to a 38 question computer-driven interview about the severity of subjective withdrawal and craving symptoms. For 9 of the 38 subjective symptoms, subjects in the NSD group had significantly ($P<.05$) lower severity of symptoms than those in the Methadone group, and for 22 other symptoms the NSD subjects had lower severity of 4 or more points. Statistical significance for those 22 symptoms was not attained because of sample sire and the variability of responses.

Table 2. *Mean severity of selected withdrawal and craving symptoms among those using the Neuro Stimulator Device versus methadone.

Symptom	Mean C N=10	Mean B N=7	Sign/	Points difference
Overall Withdrawal Symptom	13	21	.05	8
Perspiration	11	22	.03	11
Gooseflesh	6	16	.01	10
Breathing Difficulty	7	15	.05	8
Craving for Heroin	13	21	NS	8
Craving for Cocaine	4	8	.05	4

*Severity of Withdrawal and Craving symptoms is measured on a 33 points scale (From 1 to 33). low scores are favorable.

DISCUSSION

The evidence from this pilot study suggest, in a preliminary way, that the NSD maybe as effective or more effective than methadone during detoxification from opiates and in alleviating a wide variety of objective and subjective symptoms. The small sample sires and various data collection flaws limit the confidence in such preliminary results. A larger study designed to address these methodological difficulties and collect data from a larger sample is being undertaken in 1989.

AFFILIATION: Narcotic and Drug Research, Inc.[1] St. Luke's-Roosevelt Hospital Center[2]

Pavlovian Conditioning to Morphine in Opiate Abusers

David B. Newlin, Mary Beth Pretorius and Jerome H. Jaffe

Previous demonstrations of conditioned responses to opiate cues in humans have not involved opiate conditionig trials in the laboratory. This experiment was a direct application of Siegel's (1975, 1983) Pavlovian conditioning model of morphine tolerance to human opiate abusers.

We were particularly interested in the placebo response for several reasons. First, the conditioned response is pivotal to the Pavlovian model because it is the proposed mechanism of situational specificity of tolerance and other conditioning phenomena. If there were no measurable placebo response, as has often been the case in animal research, then other models are needed to account for the results (Baker & Tiffany, 1985). Demonstration of the drug-opposite response to morphine cues has been a focus for controversy in the animal literature, and human subjects allow more comprhensive measurement of the placebo response. In addition, it is possible to measure mood in humans to assess affective components of the conditioned respnse. Third, the conditioned response to opiate cues is important clinically because it may mediate relapse behavior. Finally, ethical concerns dictate that subjects in a morphine conditioning study have opiate experience. Therefore, it may prove difficult to measure the development of conditioned morphine tolerance in the laboratory because they are already tolerant to opiates. However, this previous conditioning history would not interfere with expression of the placebo response.

DESIGN

The experimental design was entirely within-subjects, with Session (1 through 7) and Trial (1 through 20) as repeated factors. Sessions were: 1) no injection, 2) morphine, 3) morphine, 4) morphine, 5) morphine, 6) placebo, and 7) nmphine. Morphine was always 20 mg i.m., and the nurse who performed the injectionwas blind. The first six sessions were all in the same distinctive laboratory environment, and the seventh session was in a different distinct &amber. The two chambers were counter-balanced between subjects, and the time of testing (a.m. vs. p.m.) was also counter-balanced.

SUBJECTS

Subjects were 12 male opiate abusers on the inpatient unit of the NIDA Addiction Research Center. Their mean age was 34.8 years, and the mean number of years of previous opiate use was 9.6 years. The subjects were generally light opiate users, and none of them were observed to experience withdrawal symptoms upon entry to the inpatient unit.

RESULTS

The heart rate response to morphine was biphasic. Morphine significantly ($p<.0001$) increased heart rate immediately after the injection, but decreased ($p<.001$) heart rate from 80 to 100 minutes later compared to a no-drug control condition. Placebo significantly ($p<.Ol$) decreased heart rate throughout the post-drug interval compared to the no-drug control. The effect of morphine on finger temperature was also biphasic; it initially decreased ($p<.05$) temperature, then increased it ($p<.05$). Morphine significantly ($p<.0001$) increased cheek temperature, and placebo increased cheek temperature ($p<.05$) during the rising morphine blood curve. Cheek temperature showed significant ($p<.001$) differentiation between the conditioned and novel environments. The results for motor activity were generally nonsignificant.

Oral temperature, MBG Euphoria, and pupil diameter showed only morphine effects. The drug and placebo effects were both significant and in the same direction for the analgesia measure ($p<.0001$) and POMS Negative Affect ($p<.001$). For static ataxia, morphine increased ($p<.01$) body sway and placebo decreased ($p<.05$) sway.

DISCUSSION

The results provided relatively strong evidence for autonomic and subjective responses to morphine placebo. The placebo response consisted primrily of decreases in heart rate and static ataxia, and increased analgesia. Placebo was dysphoric, as indicated by increased POMS Negative Affect after placebo injection.

Evidence for development of tolerance to morphine in the laboratory and differentiation of the conditioned and novel enviroments was relatively spotty, probably because of the subjets' previous opiate histories. The results indicate that morphine effects can be conditioned in the laboratory, and they correspond to some extent with Siegel's (1975, 1983) Pavlovian model. Some placebo responses were in the same direction as the drug effect, and some were opposite in direction. Further research is needed to assess the motivational significance of these conditioned responses.

AFFILIATION: NIDA Addiction Research Center, 4940 Eastern Avenue, Baltimore, MD 21224.

Effects of Tramadol in Humans: Assessment of its Abuse Potential.

Kenzie L. Preston and Donald R. Jasinski

Tramadol is an analgesic one tenth as potent as morphine. To assess its abuse potential, tramadol 75, 150 and 300 mg, morphine 15 and 30 mg, and placebo were given IM to 12 volunteer non-dependent opiate abusers. Treatments were tested under double blind conditions on consecutive days according to two 6 x 6 latin squares. Subjective, observer-rated and miotic changes were assessed prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, and 12 hours after dosing. Subjective effects were measured with ARCI subscales and a series of visual analog scale measures including drug effect, liking, drug identification, and symptoms. Observers rated subjects for drug effect, liking, and signs on a series of visual analog scales.

The onset, time to peak and duration of psychoactivity for morphine and tramadol were similar. Morphine produced typical effects including miosis and increases in ratings of drug effect, drug liking, MBG (euphoria) scale scores, and identifications as an opiate. Tramadol 300 mg was also identified as an opiate, but did not produce morphine-like subjective or behavioral effects. Tramadol also did not produce miosis. The magnitudes of the effects produced by tramadol were less than that predicted by its analgesic potency relative to morphine. The effects of tramadol 75 and 150 mg were not different from placebo. There was no evidence that tramadol produced dysphoric effects as have been shown for agonist/ antagonist opioids such as pentazocine.

We conclude that the parenteral analgesic preparations of tramadol have significantly lesser abuse potential than equianalgesic preparations of morphine.

AFFILIATION

Francis Scott Key Medical Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Acute Opioid Physical Dependence in Humans: Maximum Morphine-Naloxone Interval

Kimberly C. Kirby, Maxine L. Stitzer and Stephen J. Heishman

Acute opioid physical dependence has been demonstrated in a variety of species, but the effect of varying the temporal interval between agonist and antagonist administration has not been systematically investigated until recently. Heishman et al. (in press) showed that the minimum interval between injections necessary to observe antagonist precipitated withdrawal in humans is between 15 and 45 min. The purpose of this study was to find the maximum interval between agonist and antagonist exposure that would still allow observation of precipitated withdrawal.

METHOD

Subjects

Participants were 6 male volunteers, 33-43 yrs old, with a 7 1/2 to 31 yr history of opiate use. Their average current opiate use was approximately 4 times per week. They were not currently opiate-dependent. Participants lived on an 8-bed inpatient research unit during the study and 1-2 volunteers participated at a tune.

Procedures

Half of the subjects received morphine doses of 18mg/70kg and half received doses of 30mg/70kg. Doses were determined by conducting a naloxone challenge 6 hrs after administering the 18mg dose of morphine and looking for abstinence signs or symptoms. If abstinence signs and symptoms were not observed, the subject was assigned to the higher dose. Analysis indicated there were no significant differences between morphine dose groups. The naloxone dose of 10mg/70kg was chosen on the basis of previous work using a 6-hr agonist-to-challenge interval.

The intervals between agonist and antagonist injections were 6, 12, 24, 36, 48, 60, and 72 hrs. Each subject was exposed once to each interval in a random order. All injections were given intramuscularly under double-blind conditions. Once during each condition a placebo morphine injection occurred at one of the scheduled injection times. All morphine and placebo morphine injections occurred on the inpatient ward. Prior to naloxone injections, the volunteer was seated in an experimental room and physiological measures were given 30 min.to stabilize. Respiration rate, heart rate, blood pressure, and skin temperature were recorded continuously. Pupil photos were taken and observer and subject ratings recorded 15 min prior to the scheduled injection time and at 5, 15, 30, 45, and 60

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min post-injection. Observer measures consisted of traditional Himmelsbach signs, such as yawning, lacrimation, perspiration, and restlessness. Subject report measures included opioid and withdrawal symptom questionnaires, each containing 15 adjectives describing opioid and withdrawal symptoms. A drug effects questionnaire was also completed by the subject; this assessed global drug effects such as good and bad drug effects and general feelings of withdrawal sickness.

RESULTS

Naloxone effectively reversed residual morphine effects (e.g., pupillary constriction and reports of good drug effects) that were present at 6 and 12 hrs post morphine. By 24 hrs, morphine effects were no longer evident, and naloxone had no effect on measures designed to detect reversal of residual morphine effects.

Naloxone elevated observer ratings of abstinence at 6, 12, and 24 hrs. Ratings of abstinence were somewhat higher at the 12 hr challenge as compared to the challenge at 6 hrs, but this difference was not significant By 36 hrs, postnaloxone ratings did not differ from the prenaloxone baseline, indicating that naloxone did not precipitate an abstinence syndrome when the morphine and naloxone administrations were separated by 36 hrs or longer.

Composite subject ratings from the 15-item withdrawal symptom questionnaire agreed with observer ratings in that at 6, 12, and 24 hrs naloxone elevated ratings of abstinence . Unlike the observer ratings, the subject reported withdrawal symptoms at 6 and 12 hrs were similar, while the symptoms were significantly reduced by 24 hrs. Subjects' rating of withdrawal sickness and bad drug effect were elevated at 6 and 12 hrs, but were not significantly elevated from pre-challenge scores at 24 hrs post-morphine. Examining the individual items in the composite withdrawal symptom rating showed that at the 24 hr condition, subjects continued to give elevated ratings on items such as yawning and watery eyes, while items such as upset stomach and abdominal cramps were similar to the prenaloxone rating. This might suggest that feelings of nausea are more likely to lead subjects to identify symptoms as withdrawal sickness and/or bad drug effect than are symptoms such as yawning and watery eyes.

CONCLUSION

When these results are integrated with previous work of Heishman, we conclude that acute physical dependence effects can be observed at agonist-to-challenge intervals from 45 min to 24 hrs post-morphine. This time course suggests temporal spacing of exposures may be important in the development of physical dependence. We have begun to systematically investigate the possibility of a temporal window for acute physical dependence in our laboratory.

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Dept. of Psychia. & Beh. Science, Johns Hopkins University School of Medicine

Acute Physical Dependence in Man: Repeated Naloxone-Precipitated Withdrawal After a Single Dose of Methadone

Curtis Wright, George E. Bigelow and Maxine L. Stitzer

Opioid abstinence syndromes have been precipitated in man by naloxone administration in both clinical and experimental settings following pre-treatment with opioid agonists. Naloxone is normally without subjective or objective effect in opiate-free individuals in doses below 2 mg/kg. In contrast, naloxone has been reported to precipitate withdrawal in doses of 0.2 mg/kg after a single dose of 18-30 mg of morphine in nondependent post-addicts and in doses of 0.002 mg/kg in opioid-dependent subjects. The degree of induced naloxone sensitivity in man thus provides an estimate of acute physical dependence following the administration of opioid drugs.

In an attempt to extend-the use of this technique to single-dose studies of other opioid agonists, six non-dependent naloxone-insensitive human volunteers with a history of prior opioid abuse were pre-treated with a single dose of 30 mg methadone i.m., and their subsequent sensitivity to naloxone was determined in a double-blind experimental protocol. Each subject participated in a four-week inpatient study in which they were challenged with naloxone at 54, 30 and 6 hours after receiving methadone in a three-condition randomized-block design .

	Pre-treatment	6 hours	30 hours	54 hours
Condition 1	30 mg methadone	Placebo	Placebo	Naloxone
Condition 2	30 mg methadone	Placebo	Naloxone	Naloxone
Condition 3	30 mg methadone	Naloxone	Naloxone	Naloxone

Opioid agonist and antagonist effects were measured by a combination of self-report using subjective-effect visual analog and adjective rating scales, physiologic measures and observer ratings. Naloxone challenge was performed using a cumulative dosing technique consisting of a placebo injection followed by a series of naloxone injections (0.5 mg, 1 mg, 2 mg & 6 mg) to reduce the risk of an unpleasantly strong abstinence reaction. The subjects stopped nearly all sessions at the 0.5 mg-1.0 mg dose levels, and ratings from these levels were used in the analysis.

RESULTS

All subjects developed a mild to moderate opioid agonist effect lasting 12-24 hours after the administration of the methadone, and no subject developed an adverse drug effect. All demonstrated unequivocal naloxone sensitivity at 6, 30, and 54 hours after a single dose of methadone, with skin temperature decrease, pupillary dilation, and subjective withdrawal ratings being the most sensitive indices of naloxone sensitivity. The intensity of the reaction did not vary significantly across the three challenge times. such that the reaction was fully established at 6 hours and persisted through 54 hours with undiminished intensity.

All subjects had repeated naloxone challenges after a single dose of methadone in condition -3, and precipitated withdrawal of nearly equal intensity was observed at the 6-, 30, and 54-hour challenges under that condition.

All subjects had a 54 hour challenge in all conditions, but that challenge was the first challenge in condition 1. the second challenge in condition 2, and the third challenge in condition 3. The number of prior naloxone challenges did not affect the intensity of the reaction at the 54 hour challenge, which was nearly uniform across all these conditions.

This study establishes that a single 30 mg dose of methadone in an individual with a previous history of drug abuse induces a sensitivity to naloxone at the 0.01-0.02 mg/kg level lasting at least 54 hours, and that the intensity of this sensitivity is little affected by repeated naloxone challenge under the conditions of this study.

Supported by NIDA Grants R01 DA-03889, T32 DA-07209 & K05 DA-00050

AFFILIATION

Behavioral Pharmacology Research Unit, Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Neurolectic Correlates of Nonsteroidal Analgesia

Sean O'Connor, Allan Tasman, Nancy DePalma and Scott Zaccheo

Evoked potentials provide objective measures of the brain's response to specific stimuli. By choosing the sensory modality and controlling the intensity, timing and context of the stimulus, the EP can be analyzed for correlates of specific neural systems' responses attributable to both pathology and pharmacologic intervention. When noxious stimuli are used, the recorded waveforms are called nociceptive evoked potentials (NEPs), and are useful for studying objective concomitants of the experience of pain.

Our laboratory has developed techniques and apparatus that facilitates the collection of NEP data when the investigator is interested in responses spanning several stimulus intensities and in repeating the test at frequent intervals in order to track kinetic properties of analgesic drugs. The approach uses brief bursts of electrical current applied to the center of an upper incisor tooth surface in a way that directs the current into the tooth pulp.

Two devices are key in enabling many tests to be repeated on one subject in a way that assures the resulting NEPs can be used to detect drug effects . The first is a silastic mold custom fitted for each subject, then modified to permit stimuli to be delivered to precisely the same location on the subject's tooth in each test even if weeks apart. An 18 gauge needle is mounted on a retractable guide so that the mold can be removed and replaced with ease. The tip of the needle is modified so that a 3 mm diameter plastic cup rests squarely on the surface at the base of the tooth pulp. The needle and cup are filled with conducting electrode gel so that very little variation in contact impedance or locus is encountered. A plastic tube is also mounted on the mold to direct a stream of dry air over the tooth's surface. Thus, surface moisture which could conduct the applied current away from desired pathway is minimized.

The second device is an electronic circuit that is under the control of the computer which manages NEP data collection. The circuit generates current pulses that are precisely timed, biphasis to minimize stimulus artifact, and carefully controlled in amplitude. The pulses are also electrically isolated from all other apparatus and limited to 500 microamps in amplitude in order to assure subject safety and to preclude tissue damage. Six digitally controlled channels are available, and each channel can be preset to any one of a thousand different pulse intensities in order to collect data that span an individual's intensity/response range in any one test.

NEPs are standardized against each individual's subjective profile as a function of stimulus intensity. Using the method of ascending and descending limits, the pulse intensity marking sensory threshold is determined. Another amplitude, determined by the subject as the most painful intensity tolerable without blinking, is defined as the reference pulse. The subject is then given a series of paired pulses of which the first is always the reference pulse. The second is chosen by the operator unknown to the subject. The subject rates the subjective intensity of the second pulse as a fraction between sensory threshold and reference on a continuous dial marked "0". "1/4", "1/2", "3/4", and "1/1". A long-linear regression model of the subjective data is computed and used to determine the current intensities corresponding to 0.10,20,40 and 80 percent of the subjective response between threshold and an asymptotic response limit which is usually slightly above the reference level. These intensities are encoded into the pulse generating apparatus.

The computer then delivers a train of 50 msec long pulses at intervals uniformly distributed between 1.6 and 2.0 seconds to eliminate timed expectations by the subject. The current intensities are randomly distributed between the five active channels, and the EEG recordings are sorted by current intensity. The peak amplitude of the NEPs are checked to see that they fit the mathematical model of the subject response determined over the full stimulus range.

Pilot studies demonstrated a very good ability to provide highly repeatable results in baseline measures, both subjective and objective, taken a week apart in all subjects. The advantage of the NEP approach is that a complete block of data can be collected in seven minutes, permitting serial testing as an experimental analgesic is metabolized.

The oral presentation reviews data from double blind, placebo controlled, 3 way crossover design testing the analgesic effect of two forms of ibuprofen; the standard pill preparation and a liquid form that is more rapidly absorbed by the gut. The results demonstrate that analgesic effect is clearly differentiated from placebo effect in the first hour of testing by significant decreases in NEP amplitude. Moreover, a kinetic model of the data detects the more rapid onset of analgesia afforded by the liquid ibuprofen.

The cost of developing the apparatus and techniques and of conducting ibuprofen studies was funded by the Upjohn Corporation.

AFFILIATION:

University of Connecticut School of Medicine, Farmington, CT 06032

Oral Naloxone Treatment of Narcotic Induced Constipation: Dose Response

Joan A. Culpepper-Morgan, Charles Inturissi, Russel Portnoy and Mary Jeanne Kreek

Opioid agonists are known to cause constipation in individuals who require chronic administration of these drugs for pain or methadone maintainence. This symptom is often resistant to treatment with conventional laxatives. Naloxone, a specific opioid antagonist, reverses all opioid effects and induces narcotic withdrawal when given intravenously. However, Kreek et.al. observed that methadone maintained patients who were given oral naloxone underwent profound withdrawal that was mostly limited to the gastrointestinal tract (GI) (1). This was felt to be due to the limited systemic bioavailability of naloxone which is inactivated by glucuronidation in the 3-OH position on first pass through the liver (2).

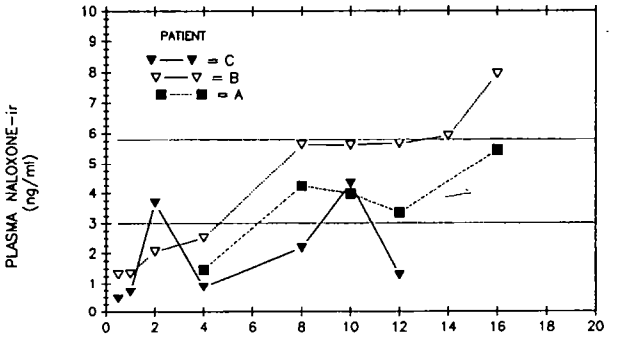
We have examined the ability of orally administered opioid agonists to delay GI transit in the guinea pig and found mu and kappa agonists to be equipotent by this route (3). We have recently reported on the effects of orally administered narcotic antagonists in the same guinea pig model. We have determined that, despite limited systemic bioavailability, orally administered naloxone can reverse opioid agonist induced slowing of GI transit (4). Although, a primary gut wall site of action is implied the low levels of plasma naloxone that can be measured by radioimmunoassay should parallel the local gut wall concentration.

Therefore the aim of the present study was to determine if orally administered naloxone can reverse. narcotic induced constipation without causing systemic withdrawal or the recrudescence of pain. A related aim was to determine if efficacy or adverse reaction could be related to plasma naloxone levels.

Three patients who required chronic treatment with opiates for the relief of pain and who were unable to have a spontaneous bowel movement without the use of laxatives were studied. Patients were given a single daily oral dose of naloxone in a titration study design. Doses ranged from 0.5 mg to 16 mg. Plasma levels of naloxone were measured by radioimmunoassay on extracted (active naloxone only) and unextracted (active naloxone + inactive glucoronide) plasma (5).

Extracted plasma naloxone levels were < 10% of unextracted levels. The peak naloxone plasma levels for each dose administered are shown below. Patient A had a dose related improvement in spontaneous laxation and stool consistency and

MAXIMUM EXTRACTED PLASMA NALOXONE LEVELS vs DOSE IN 3 PATIENTS



no adverse reactions. Patient B had symptoms of withdrawal at doses greater than 12 mg and crampy abdominal pain. At doses of 8-10 mg patient C had symptoms of pending laxation but did not have a spontaneous bowel movement or symtoms of withdrawal.

We conclude, that first pass biotransformation of orally administered naloxone significantly reduces the amount of active compound that is delivered to the systemic circulation. Some patients may have transit that is to slow to obtain steady gut wall levels of drug, such as patient C. Steady state naloxone levels betwen 3 and 6 ng/ml seem to be associated with spontaneous laxation without narcotic withdrawal. Lastly, Albeck et. al. have demonstrated that the pharmacokinetic profile of naloxone may indicate that it has a prolonged terminal half life of up to 120 minutes (6). Therefore, future evaluation of oral naloxone for the treatment of narcotic induced constipation should titrate the patients dose using single doses less than 12 mg going on to multiple doses of 12 mg or less at intervals of 6-12 hours. These studies are currently in progress

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AFFILIATIONS: Rockefeller University, Cornell University Medical College, Memorial Sloan Kettering Cancer Center, and Norwalk Hospital.

HIV Risk Behavior: Antisocial Personality Disorder, Drug Use Patterns, and Sexual Behavior Among Methadone Maintenance Admissions

Dušan Nolimal and Thomas J. Crowley

The literature suggests an association between Antisocial Personality Disorder (ASPD) and drug use. The risk of Human Immunodeficiency Virus (HIV) infection has been associated with intravenous drug use, equipment-sharing and high-risk sexual activities. Intravenous drug users (IVDUs) may not be a homogenous population. They might vary considerably in their risk for HIV contraction and transmission. These findings led the authors to hypothesize that IVDUs with ASPD are more likely to be involved in HIV-risk behaviors, and have more problems with alcohol and non-opioid drugs than non-ASPD IVDUs.

Shortened NIMH Diagnostic Interview Schedule (DIS-III) and HIV-Risk Behavior Interviews were given to 52 consecutive methadone maintenance admissions (MMAs). One interviewer completed each evaluation the first day of admission. The validity of the self-report data was assessed through consistency controls of comparing the sample data with other relevant sources. Data were scored for lifetime and/or last year ASPD, number of ASPD signs and symptoms, alcohol abuse/dependence, non-opioid drug abuse/dependence, injection-equipment sharing and cleaning, and sexual behavior. T-test, Chi-square, correlation and regression statistics were used to compare ASPD with the non-ASPD group.

There were-no significant differences in sex, age, race, marital status, education, employment, monthly income and number of previous treatments between the groups. Fifty MMAs (96%) were diagnosed as lifetime opioid IVDUs. Forty-four (85%) used drugs intravenously and 29 (56%) shared equipment 12 months prior to the admission. All studied subjects but two were sexually active in the previous year. No one was HIV seropositive at the time of admission.

DIS-III diagnosis indicated that 40% of the MMAs met the criteria for a definite diagnosis of lifetime ASPD. Antisocial MMAs were more likely to have an earlier criminal record, sexual relations, drug use and intravenous drug use. They reported significantly more lifetime and last year non-opioid drug problems. related to alcohol (p=.01) and cocaine (p=.03) than non-antisocial. The ASPD patients had more injection-sharing partners (mean=2.6 vs. 1.0,

p=.0009). and shared equipment more often (p=.01), than non-ASPD patients. Also, the ASPD group had more sex partners (mean=6.1 vs. 1.6. p=.01). The relationship between ASPD and HIV-risk behavior was strongest for lifetime diagnosis of promiscuity (p=.0002) and prostitution (p=.0004). There were no significant differences in frequency of daily intravenous drug use per year, equipment cleaning when shared, frequency of daily sexual activities per year and condom use patterns between the groups. Number of ASPD signs and symptoms across the entire sample significantly correlated with a frequency of HIV-risk behavior (r=.378 for no. of sharing partners; r=.369 for no. of sex partners). The 15% of MMAs with the highest number of antisocial signs accounted for 49% of all last year sex partners and for 22% of all last year equipment-sharing partners. The number of sharing partners correlated significantly (r=.45) with the number of sex partners.

The study indicates that ASPD among MMAs is associated with more HIV-risk behaviors and conditions, including a high prevalence of equipment-sharing, sex partners, and alcohol and cocaine problems. The number of antisocial signs and symptoms correlates with the frequency of HIV-risk behaviors. Promiscuity and prostitution are highly associated with ASPD and significantly increase the risk for HIV's spread. Individuals with multiple equipment-sharing partners are also likely to have multiple sex partners. Overall, there is a wide variation in levels of HIV risk behavior among MMAs, but individuals with a high number of ASPD lifetime features are at high risk to contract and transmit HIV.

AUTHORS:

Dušan Nolimal, M. D.
Thomas J. Crowley, M. D.
Department of Psychiatry
University of Colorado School of Medicine
4200 East 9th Avenue, C268
Denver, Colorado 80262

Primary Medical Care for IVDU's: A Model for Future Care

Richard S. Schottenfeld and Patrick G. O'Connor

BACKGROUND: IVDU's have traditionally faced problems gaining access to medical care and drug abuse treatment, obtaining comprehensive medical and drug abuse treatment, and receiving coordinated care from multiple providers. In New Haven, fewer than 1/4 are in drug abuse treatment programs, and only 1/6 IVDU's with AIDS had ever been in treatment. This is in contrast to the Netherlands where low threshold treatment programs serve virtually all addicts. What has been the impact of HIV infection and AIDS in this population? What new services or approaches to treatment need to be developed to address these problems? What are the advantages and disadvantages of providing comprehensive, integrated primary medical care and drug abuse treatment in one program?

RESULTS Because methadone regulations mandate admission and annual physical examinations, we had previously developed a small, primary medical care facility (Central Medical Unit, CMU) to provide physical examinations and limited primary medical care as part of our multi-modality treatment program. In July, 1988, additional funds were made available, allowing us to add a--full-time internist, nurse practitioner and medical.. technician to the previous staffing (part-time internist, nurse practitioner, physician's associate, AIDS educator, and secretary).

RESULTS CMU now provides 1) primary medical care to IVDU's enrolled in substance abuse treatment programs, 2) opiate detoxification and introduction to drug abuse treatment, 3) consultation and teaching about drug abuse to the Primary Care Clinics (PCC) and medical service of Yale-New Haven Hospital, and 4) medical support to a medical van used in a NIDA-sponsored comprehensive AIDS outreach and prevention project.

During the first four months of full staffing, there were 1392 patient visits. A sample of 146 consecutive unduplicated, patients revealed that 72% were Caucasian and

63% male, with an age range of 21 to 71 years. 83 patients were referred from methadone maintenance and 63 from drug-free treatment programs for opiate, cocaine or alcohol disorders. Screening and preventive health care services were the primary reason for visits in 42% (62/146) of these patients. The remaining 84 patients were referred for evaluation and management of active medical problems: 21% (18/84) had HIV related illness and 26% (22/841 suffered from other direct complications of intravenous drug use (e.g., hepatitis, cellulitis). A wide range of general medical problems, including hypertension, diabetes, and common infections were cared for as well.

DISCUSSION: Benefits of the expansion of CMU include: 1) better coordination of care when patients are hospitalized, facilitating hospital treatment, discharge planning and follow-up; 2) better compliance with treatment--CMU clinicians encourage patients to return to drug abuse treatment programs, and clinicians in drug abuse treatment reenforce the need for continued medical treatment; 3) improved ability of medical providers to care for IVDU's. Additional advantages to providing coordinated and integrated primary medical care and drug abuse treatment in one facility are likely: 4) CMU can function as a low threshold, entry level program for IVDU's who typically drop out of or never utilize drug abuse treatment programs because of stringent program rules and insistence on abstinence--CMU would not, however, prescribe opiates, sedatives, or needles and syringes that would enable continued drug use; 5) CMU will identify cohorts of IVDU's with HIV that can be used in clinical trials of therapeutic agents, thus improving the medical care of IVDU's and contributing to scientific advance; 6) CMU can develop service modules for women IVDU's (Women's Health Services to address prenatal, post-partum care, battering, prostitution, sexually-transmitted diseases, and parenting issues) and for children of IVDU's (Pediatric Clinic to address child development and adequacy of medical care).

A potential disadvantage of combining medical and drug abuse treatment services in one setting is the possibility that neither treatment will be provided well, thus forestalling adequate care. To avoid this pitfall, programs need to define clearly which services they can provide and when they need to refer to specialty clinics.

Because primary medical care facilities are available in most communities in the U.S., developing drug abuse treatment services in these facilities may be a cost effective alternative to developing new drug abuse treatment services. (Yale University School of Medicine)

HIV-Infected IV Drug Users in Methadone Treatment: Outcome and Psychological Correlates-A Preliminary Report

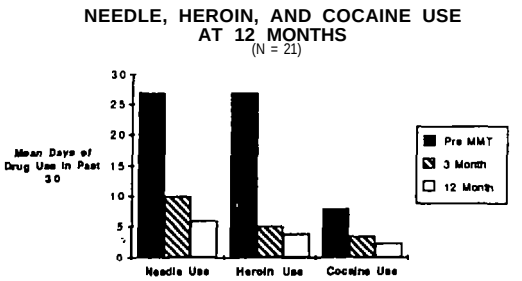
Steven L. Batki, James L. Sorensen, David R. Gibson and Peg Maude-Griffin

The effectiveness of methadone maintenance treatment (MMT) for intravenous drug users (IVDUs) with HIV infection is crucial in limiting the spread of AIDS (1). This study examined MMT effectiveness and the role of psychiatric symptoms in treatment outcome among HIV-infected IVDUs. Psychological distress was studied because it is common in HIV-infected IVDUs and because of evidence that depression and other psychiatric symptoms predict poor drug abuse treatment outcome (2). The role of cocaine was examined because of the importance of cocaine use in IVDUs with AIDS (3).

METHOD: Subjects were the first 42 consecutive HIV-infected IVDUs admitted to MMT at the Program for AIDS Counseling and Education (PACE) at San Francisco General Hospital Substance Abuse Services. Measures were prospectively taken at entry, and after three months and 12 months of MMT. IV drug use measures included the Addiction Severity Index (ASI), urine tests, and skin examination for needle marks. Psychological measures included the Beck Depression Inventory (BDI), the Beck Hopelessness Inventory (BHI), and the depression and suicidality items from the ASI. Two thirds of the subjects were male; the majority were black or hispanic. Mean age was 37.5 years with a mean of 12 years of heroin use. About half of the subjects had previous MMT. 62% were heterosexual. Most subjects had some symptoms of HIV infection.

RESULTS: Subjects demonstrated significant decreases in IV drug use and heroin use in the first three months of MMT. Cocaine use decreased, but not significantly. Urine testing and skin puncture examination confirmed the direction of these changes. Clinical improvement was maintained at the 12-month follow-up (4) (Fig. 1).

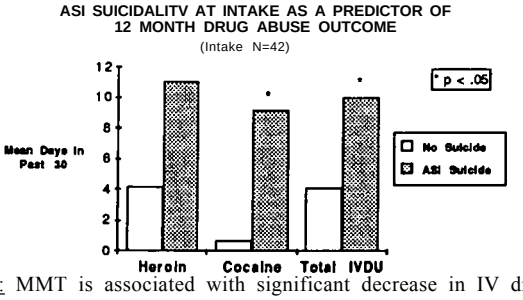
Figure 1



Subjects had high levels of psychological distress at entry. 60% had moderate to high levels of depression on the BDI. BDI scores did not decline significantly during MMT; mean scores consistently exceeded moderate levels. The Beck Hopelessness Inventory measures pessimism and is a useful predictor of suicidality. 57% of subjects had moderate or higher levels of hopelessness on the BHI. As with the BDI, there was no significant decrease in hopelessness scores during treatment. Items from the ASI were also used to assess psychological distress. More than 72% of subjects reported depression in the month prior to entry. Half of the depressed patients, or nearly 37% of the entire group, reported thoughts of suicide, and a strikingly high 13% of patients reported making a suicide attempt in the month prior to entry.

Patients who reported initial depression on the ASI had almost six times the number of days of IV drug use at 12 months ($p<.03$) than patients who denied depression at entry into MMT. Depression at intake also predicted later cocaine use. Subjects with intake BDI scores over 30 had 10 times the number of days of cocaine use at 12 months than subjects with low BDIs at entry. Among the measures of distress, suicidality at intake appeared to be the most consistent predictor of future drug use outcome. Patients with suicidality at intake reported almost three time as many days of heroin use, 16 times as many days of cocaine use, and nearly three times as many days of total IV drug use at 12 months of treatment than patients with no suicidality (Fig. 2). Cocaine abuse at admission to MMT was an even more robust predictor of MMT outcome than were measures of psychological distress. Patients who reported cocaine use at intake had significantly more days of heroin, cocaine, and total IVDU at 12 months than those patients who denied pre-admission cocaine use.

Figure 2



DISCUSSION: MMT is associated with significant decrease in IV drug use in HIV-infected patients despite the prevalence of psychological distress at entry and throughout the first year of MMT. Higher levels of depression, hopelessness, suicidality, and cocaine use at intake appear to be predictive of poor treatment outcome at 12 months of MMT. This is the first study known to us to prospectively examine MMT outcome in HIV-infected IVDUs. These early results show that MMT is effective even in this severely distressed group of patients. Further studies should determine whether early assessment and treatment of depression and cocaine use among HIV-infected IVDUs will improve treatment outcome.

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Mode of HIV Transmission Among Seroconverted Intravenous Drug Users (IVDUs): 1987 and 1988 Cohort Study

Tooru Nemoto, Lawrence S. Brown, Jr., Beny J. Primm, Kenneth Foster and Alvin Chu

OBJECTIVE

To investigate the mode of HIV transmission among IVDUs who were enrolled into the methadone maintenance programs in New York City, especially focusing on drug and sexual behaviors among seroconverted IVDUs.

METHODS

A total 440 subjects (218 in 1987 and 222 in 1988) were recruited from the methadone maintenance programs in Manhattan and Brooklyn, New York City. The characteristics of subjects were: 62% males and 38% females; 51% Blacks, 42% Hispanics, 7% Whites and others; Mean age = 33 years. Thirty-seven percent and 77% of the subjects were recruited from the newly admitted patients in 1987 and 1988, respectively. There was no significant difference between the subjects and patient population of the methadone programs in age, sex, and ethnicity. After informed consent, a standardized questionnaire was administered by trained interviewers. Also, blood was collected and tested for HIV antibody by ELISA and Western Blot techniques.

RESULTS

The overall HIV infection rate was 60% in 1987 and 52% in 1988. No significant association was found between HIV serostatus and ethnicity, sex, type of admission, and levels of education both in the 1987 and 1988 cohort groups. However, there vere significant associations between HIV serostatus and age groups in 1987 ($p < .10$) and in 1988 ($p < .01$), that is, the older groups were more likely to be found seropositlve. Among the 1987 cohort group, 25 subjects reported that they had previously tested for HIV serostatus. Two reported HIV positive and 23 had HIV negative. Nineteen out of 23 (83%) who reported previous HIV seronegatlve status, were found to test HIV seropositive. Among the 1988 cohort group, 40 subjects reported that they had previously tested for HIV serostatus and were HIV negative.

Twenty-three out of 40 (57%) who reported previous HIV seronegative status, were found to test HIV seropositive.

In the 1987 cohort group, no significant difference was found between the seroconverted and remaining HIV seronegative subjects in age, sex, ethnicity, levels of education, locations of clinics, and drug and sexual behaviors. However, in the 1988 cohort group, significant differences were found between the seroconverted and remaining HIV seronegative subjects on the frequency of sharing needles during the past 5 years ($p=.003$), the frequency of sharing cookers during the past 5 years ($p=.006$), the number of needle sharing partners during the past 5 years ($p=.008$), the frequency of using shooting galleries during the part 5 years ($p=.003$), and the frequency of cleaning needles during the past 5 years ($p=.013$). No significant difference was found in these drug behaviors during the past 12 months. The type of drugs (heroin, cocaine, and marijuana) and the way of drug taking behaviors (intravenous, skin-pop, smoking, snorting) were examined. In the 1988 cohort group, the seroconverted subjects reported significantly higher frequencies of using speedball than those who remaining seronegatlve ($p=.02$). In the 1987 cohort group, all subjects who remained seronegative reported they stopped needle sharing and reduced number of sex partners.

DISCUSSION

The study was different from the cross-sectional studies in HIV seroprevalence among IVDUs which reported the association between HIV serostatus and demographic variables, and drug and sexual behaviors. This study mainly focused on the drug and sexual behaviors, and demographic variables which differentiated the seroconverted IVDUs from the remaining seronegatlve IVDUs in the 1987 and 1988 cohort groups. The limitations of self-report by subjects on HIV serostatus, and drug and sexual behaviors should be considered. However, this study clearly demonstrated, especially among the 1988 cohort group, that needle sharing behaviors and needle sharing at shooting galleries during the past 5 years significantly differentiated the seroconverted IVDUs from the remaining seronegative IVDUs. Also, the seroconverters reported significantly higher frequencies of using speedball during the past 12 months. This study supported the past research findings that HIV infection among IVDUs was strongly associated with needle sharing behavior, needle sharing at shooting galleries, and IV cocaine use. Furthermore, this study demonstrated that these high risk behaviors for HIV infection discriminated the seroconverted IVDUs from the remaining seronegative IVDUs. In order to prevent HIV Infection among IVDUs, more effective interventions to educate IVDUs to stop needle sharing and engaging in high risk drug behaviors are needed.

AFFILIATIONS: Addiction Research and Treatment Corporation, Brooklyn, NY and Harlem Hospital, College of Physicians and Surgeons, New York, NY.

Developmental Decline in Infants Born to HIV-Infected Intravenous Drug-Using Mothers

R. Kletter, R. J. Jeremy, C. Rumsey, P. Weintrub and M. Cowan

OBJECTIVE

To chart prospectively the course of development of infants exposed in utero to the human immunodeficiency virus (HIV), and compare infants who have become infected with those who have not.

METHODOLOGY

Participants

Three groups of infants born to intravenous (IV) drug-using women:

Group I: Uninfected infants of uninfected mothers (Control; N = 13)
Group II: Infected infants of HIV-infected mothers (Infected; N = 9)
Group III: Uninfected infants of HIV-infected mothers (Uninfected; N = 12)

The infants are still being followed and new ones enrolled into the ongoing study.

Instruments and Procedures

Infants are assessed every 3 months on the Bayley Scales of Infant Development. The examiners are "blinded" about the mothers' and the infants' HIV infection status.

Analyses

The Bayley exam yields standardized scores of infants' mental and motor performance, the Mental and the Psychomotor Development Indices (MDI and PDI, respectively), with a mean of 100 and a standard deviation (SD) of 16 for each of monthly ages between 3 and 30 months.

We compared the scores of the three groups of infants at each of the ages of 3, 6, 9 and 12 months (there are still too few data at the older ages).

RESULTS

1. Mental Development. The Infected infants showed declining MDI scores with age between 3 and 12 months, in contrast to both non-infected groups which did not change significantly. However, while the Control group continued to perform near normal MDI level, the Uninfected group continued to score about one standard deviation lower.

2. Motor Development. The Infected infants declined in their PDI scores. In contrast, both non-infected groups continued to perform near normal.

DISCUSSION

We found that during the first year of life, the mental and motor development of HIV-infected infants is slowing down, as indicated by decreasing MDI and PDI scores.

However, the two non-infected groups differed significantly in their typical level of mental functioning during the first year of life. While the MDI scores of the Control infants stayed at around 100, which is average for normal infants, the scores of the Uninfected ones stayed about one standard deviation below the average for normal infants. Thus, the Control infants behaved during the first year of life as expected of average normal infants. In contrast, both groups of infants of HIV-positive mothers diverged from normal, but in different ways. Those who became infected with HIV (Infected) declined with age from average to poor, while those who somehow avoided becoming infected in utero (Uninfected) did not decline significantly but, instead, functioned poorly all along since early infancy.

The Uninfected infants, like the Infected ones, showed problematic mental functioning (but of a different pattern), but, like the Control infants, their gross motor performance continued about average.

AUTHORS

R. Kletter - BAART-FACET, San Francisco, CA
R.J. Jeremy, C. Rumsey, P. Weintrub and M. Cowan - University of California, San Francisco, CA

Does Fear of AIDS Affect Behavior of Addicts?

Richard S. Schottenfeld, Stephanie S. O'Malley and Katurah Abdul-Salaam

BACKGROUND: How successful are outreach and prevention activities in changing the high risk behaviors of intravenous drug users (IVDU's)? Are addicts reducing needle sharing or intravenous use of drugs?

Pessimism about the possibility of changing the behavior of addicts is often expressed. Weinberg and Murray (1987), for example, write that "[p]ast attempts to reach the drug-abusing population through education or motivation have been unsatisfactory ....Often alienated and self-destructive, drug users have not been amenable to traditional approaches to encourage behavioral change."

Studies to date report contradictory results. Evaluation of outreach programs in Baltimore and Sacramento support pessimism: although knowledge about HIV transmission and ways to prevent the spread of HIV increased, there were no significant changes in needle sharing. Self-reported increases in the use of sterile injection equipment, increased use of bleach by IVDU's, and increased use of needle exchange programs in some countries, however, suggests that addicts are changing their high risk behaviors. (DesJarlais and Friedman, 1988) In New Haven, we were struck by reports from addicts that they were avoiding intravenous drug use in an effort to reduce their risk for contracting HIV infection.

METHODS: To evaluate whether opiate addicts in New Haven have changed behaviors as a result of AIDS, we reviewed the records of all opiate addicts seeking treatment in similar time periods in 1986 and 1988 to determine self-reports of route of drug administration. To determine the validity of self-reports, we compared findings on admission physical examinations to self-reports for those who entered treatment.

RESULTS: Opiate addicts evaluated in 1986 (n=60) did not differ significantly from those evaluated in 1988 (n=82)

with regard to demographic factors, years of opiate use, use of cocaine, or Beck Depression scores. In 1986, 52/60 (86%) of addicts reported current IV use, 4/60 (7%) reported past use but none in the preceding year, and 4/60 reported never using IV. In 1988, however, 32/82 (39%) reported no current IV use--18/82 (22%) reported never using IV and 14/82 reported switching from IV to intranasal, inhalation or oral use. (ChiSquare=11.368, df=2, p=.003). Current IV 'users had used opiates longer (mean 11.3 years) compared to those not currently using IV (mean 8.4 years, t=2.20, df=65, p<.03). There was a trend for cocaine use to be associated with current IV administration: 55% of current IV users also used cocaine, compared to 37% of those not currently using IV (Chi Square = 3.3, df=1, p=.07).

Fresh track marks were recorded in the physical examinations of 91% of self-reported current IV users (40/44), 60% of self-reported past only IV users (3/5), and 14% of self-reported never used IV (1/7). 25% (2/8) of addicts initially reporting no IV use and 44% (4/9) of addicts initially reporting past use only reported current IV use at the time of physical examination. 9% (4/43) of addicts initially reporting current IV use reported either part use only (3/43) or never using IV (1/43) at the time of physical examination.

DISCUSSION: Self-reported IV use of drugs declined significantly between 1986 and 1988 in opiate addicts seeking drug abuse treatment, possibly as a result of extensive AIDS outreach efforts and the increasingly visible impact of HIV infection in IVDU's. By 1988, 1/6 addicts reported having discontinued IV use and more than 1/5 reported never using IV.

In this study, self-reports of not using IV were found to be quite unreliable, casting some doubt on the validity of the findings. Addicts appear to bias their reports toward what is socially acceptable or. personally desirable behavior. Although addicts attributed their avoidance of intravenous drug use to concern about AIDS, changes in route of administration may have resulted in part from increased purity of street heroin, facilitating heroin smoking or intranasal use--purity of confiscated street heroin in Connecticut averaged 50-60% in 1988, considerably higher than in past years. The finding of significantly less intravenous drug use in treatment-seeking addicts in New Raven may also not be generalizable to non-treatment seeking addicts. 94% of addicts completing a health department survey in 1988 reported current IV use, and 71% reported needle-sharing. (Yale University School of Medicine)

Demographic, Behavioral and Clinical Features of HIV Infection in NYC Intravenous Drug Abusers

L. S. Brown, Jr., A. Chu, T. Nemoto and B. J. Primm

INTRODUCTION

Intravenous (iv) drug abuse continues to be pivotal in the prevalence of the acquired immunodeficiency syndrome (AIDS) among women, children, and ethnic/racial minorities. Despite this undeniable impact of iv drug abuse on HIV disease in the United States, important gaps exist in knowledge about the sexual behaviors of iv drug abusers and even fewer reports are available of the clinical features of HIV disease in iv drug abusers prior to meeting CDC criteria for AIDS. The current study presents an examination of the prevalence of HIV infection and clinical HIV disease in association with demographic, socioeconomic, and behavioral factors in a population of iv drug abusers.

PATIENTS and METHODS

From June *to* September 1985, we recruited 318 male and 136 female patients scheduled for physical examination in selected methadone maintenance clinics in Brooklyn and Manhattan of New York City. Minimal eligibility included an age of 18 years and at least one year of opiate dependence. Ages ranged from 19 to 62 yrs. with a mean age of 35±0.35 (SEM) years; the ethnic/racial distribution was 48.9% black, 42.5% Hispanic, and 8.6% white; and 46% of the study subjects were enrolled from Brooklyn clinics. Drug use ranged from 1 to 38 years with a mean of 12.3±0.34 and drug treatment enrollment ranged from 0.7 to 152 months with a mean of 23±1.4 months. The study population represented 96% of the patients eligible for participation.

Trained interviewers administered a standardized questionnaire regarding demographic and socioeconomic status, drug use patterns and sexual behaviors. Medical staff performed medical histories, physical examination, and phlebotomy.

Laboratory evaluatlon included a complete blood count, serum electrolytes, liver function tests, hepatitis B serology by enzyme immunoassay (Abbott) and syphilis serology by rapid plasma reagin. We examined collected sera for HIV-l antibody

using an ELISA technique (Genetic Systems). Repeatedly positive specimens by ELISA were confirmed using the Western blot technique (Dupont). Sera with p24 and/or gp41 bands on Western blot were considered positive. Sufficient information was available to categorize 307 subjects with a modified version of the Centers for Disease Control (CDC) staging classification for HIV infections. The modification entailed placing persons, who were not found to have any clinical or serological evidence of HIV infection, into CDC stage 0. Using the SPSS/PC+ statistical package, univariate measures were used to analyze collected data.

RESULTS

Serological evidence for HIV infection was found in 60.6% of the study population. Nineteen percent of 307 subjects were in Group III or Group IV of the CDC classification system for HIV-associated infections. While the HIV infection rate was greater among females, ethnic/racial minorities, subjects aged 25-30 years, and those who reside in Manhattan, these trends were not significant. Subjects who attained higher educational levels, who resided with their spouse and/or children, who were married, and those who admitted to receiving income from legitimate employment had lower rates of infection. The HIV seroinfection rate did not differ significantly by parameters of alcohol or tobacco use. All subjects admitted to the use of intravenous drugs and 92% admitted to polydrug use. Frequent use of IV drugs (p=.016), duration of drug use (p.=050), and duration of drug treatment enrollment (p=.038) and daily methadone dose (p=.001) were significantly associated with HIV seroinfection status.

HIV-infected subjects had significantly lower total white blood cell counts (p=.001), serum hematocrits (p=.006), mean corpuscular volumes (p=.018), absolute platelet counts (p=.018), and serum albumin levels (p=.001). Serum globulin levels were significantly (p=.001) higher in HIV-infected subjects. The dally methadone dose was significantly associated (p=.005) with CDC HIV stage, from a mean 45.5±2.3 mgs. in Group 0 subjects to 34.3±2.8 mgs. in Group IV subjects. Demographic and socioeconomic variables did not significantly differentiate seropositive subjects from seronegatlve subjects. Only behavioral factors (p=.001) and clinical indicators (p=.003) were found to distinguish the HIV-infected from the non-infected and CDC HIV stage.

CONCLUSION

These findings strongly support aggressive efforts to reduce parenteral drug use and enroll intravenous drug users into effective drug treatment.

AFFILIATIONS: Division of Research and Medical Affairs, Addiction Research and Treatment Corporation, Brooklyn, NY and Harlem Hospital, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY.

Psychiatric Symptoms in HIV Test Consenters and Refusers

George Woody, David S. Metzger, A. Thomas McLellan Charles P. O'Brien and Domenic DiPhilipis

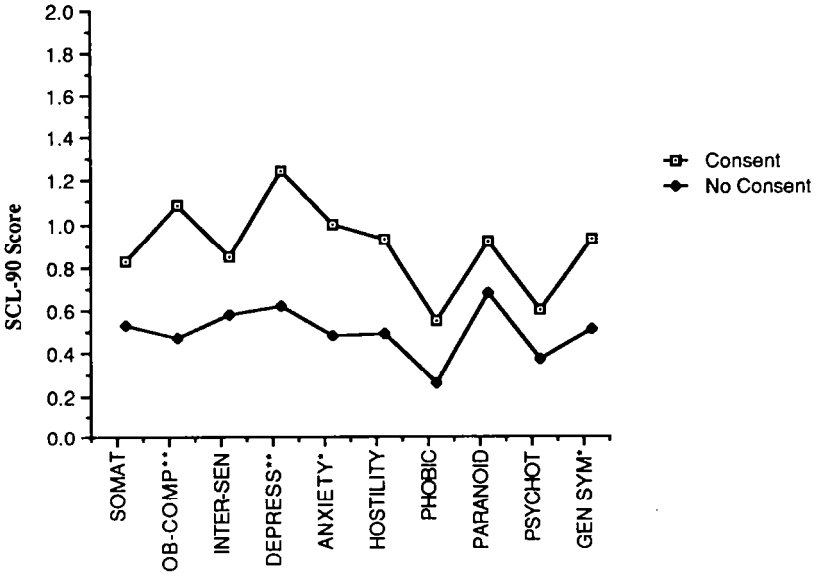
Fifty five methadone maintained opiate addicts were randomly selected to participate in a pilot study of HIV testing and high risk behavior. All agreed to complete an interview and questionnaire regarding high risk sexual and drug use behavior. Thirty three also agreed to be tested for the HIV virus and only one was positive. Five of those who refused said they had been tested within the last year and were negative.

Amounts of sexual and drug-using high risk behavior reported did not differ among those who agreed to be tested and those who declined. Psychiatric symptoms, however, were significantly different between the two groups. Those who agreed to be tested had more frequent and intense symptoms of anxiety and depression than those who declined. This difference is best reflected in the SCL-90 profiles of the two groups, as seen in the graph below.

This pilot study suggests that the level of seropositivity among methadone maintained veterans at the Philadelphia VAMC is lower than that reported from other nearby cities. It also suggests that patients who refuse testing have similar amounts of high risk behaviors than those who agree, but are less psychiatrically symptomatic. These higher symptom levels in those who agree to be tested may be a reflection of underlying anxiety., which could be attributed to characterological factors and (or) actual participation in high risk behaviors.

One implication of these findings is that IVDU's who argee to be tested for HIV may be more susceptable to the development of psychiatric disorders than those who decline. This could be particularly relevant if they test positive. Another implication is that raising anxiety levels about the potential for infection among those who decline may improve compliance with HIV testing.

XL-90 Subscale Scores by HIV Test Consent



Center for Studies on Addiction
University of Pennsylvania / Philadelphia VA Medical Center

Addict Beliefs About Access to HIV Test Results

Donald A. Calsyn, George Freeman, Jr., Andrew J. Saxon and Stephen Whittaker

Characteristics of IVDU's who sought out HIV antibody testing and their beliefs about who should have access to HIV test results were examined in 186 male and 87 female IVDU's in treatment. Sample demographics were: age (x=38.4, σ=7.4)! white 69.9, black 23.8, other 4.8%. Those who had sought out HIV antibody testing at an alternate site (N = 107) were younger (x=37.0, σ=7.2) and less likely to be employed (19%) compared to those not seeking the test (N=160; x̄=39.4, σ=7.4, t=2.64, p<.01; 34%, x^2 =7.0, p<.01). There were no education, race or sex differences. Test seekers had lived in current residence fewer months (x=19.0 σ=3 3.2 vs x=29.8, σ=41.1, t=2.28, p<.05) and had been in treatment fewer months in the past 5 years (x=22.8, σ=18.2, vs x=32.2, σ=22.8, t=3.6, p<.001). Test seekers had more needle sharing partners in the past year (x=6.26, σ=15.28 vs x=2.92, σ=8.70, t=2.25, p<.05), but there were no differences on sexual behavior variables. Most subjects believed the individual tested (91.8%), his/her physician (92.1%) and sexual partner (90.6%) should have access to test results. Many felt public health agencies (57.9%). health workers caring for the individual (60.3%), laboratory workers (52.1) and the individual's counselor (46.4%) should have access. Most felt employers (16.5%), insurance companies (21.8%) and non-public health government agencies (21.8%) should not have access. Subjects who had already had HIV testing were more likely to feel their counselor (55%) and health care workers (69%) should have access as comparedtothosenottested (41%, x^2=5.4, p<.05; 54%, x^2=5.9, p<.05). Those using drugs IV in the past 30 days (N = 130) were more likely to feel government health departments (65% vs 51%, x^2=5.3, p<.05) should have access. Of those using IV drugs in past 30 days needle sharers (N=54) were less likely to want public health agencies (56%) or sex partners (85%) to have access compared to non needle sharers (N=76; 72%, x^2=3.9, p<.05; 99%, x^2=8.9, p<.01). Subjects who knew someone with AIDS (N=75) were less likely to feel insurance companies (13%) and laboratory workers (42%) should have access as compared to those not knowing someone with AIDS (N=194, 34%, x^2=4.6, p<.05; 56%, x^2=4.0, p<.05). Addicts seem willing to share HIV test results with appropriate people but seem too willing to give results to those who have no need or such information.

AFFILIATIONS: Veterans Affairs Medical Center and Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle

Needle Obtainment and Cleaning Habits of Addicts

Adrew J. Saxon, Donald A. Calsyn, Stephen Whit-taker and George Freeman, Jr.

Extensive personal interviews conducted during 1988 examined the needle sharing, obtainment, and cleaning habits of 273 in treatment IV drug users in Washington state where injection equipment can be legally purchased in a drug store. HIV antibody testing done on 169 showed only 2(1.2%) as HIV +. The sample was 68.1% male, 69.9% white and 23.8% black with a mean age of 38.4. Primary drugs of abuse were opiates 77.1%, cocaine 11.4%, amphetamines 1.8%, ethanol 6.3%. In the prior year 68.5% had shared needles, but only 19.9% had shared in the past month. For those sharing the mean number of sharing partners in the past 30 days was 1.96. Younger age was associated with more sharing while gender and race were unrelated to sharing. Mean number of sharing partners in past year (2.3 $\pm$ 8.9 S.D.) was less for subjects in treatment longer than 6 months (N = 132) as compared to those in treatment 6 months or less (N = 138, x partners = 6.3 $\pm$ 14.1 S.D.; t=2.81, p<.01). Subjects admitting to recent IV cocaine use (N=92) share with more people in the past year (x=7.9 $\pm$ 18.0 S.D.) than those admitting to other IV drug use (N=40, x = 1.8 $\pm$ 1.8 S.D.; t =3.21, p<.002). Subjects ranked buying needles in a drug store as the most common obtainment method. Getting needles on the street and borrowing from a friend were ranked second and third. Those using IV drugs in the past year who identified drug store purchase as the most frequent obtainment method (N= 110) share less frequently (x= 14.4% $\pm$ 24.5% S.D. of injection episodes) than those getting needles from dealers, on the street or from friends (N =48, x=26.2% ± 37.4% S.D. of injection episodes; t =2.32, p<.05). When reusin needles 94.6% clean with water, 57.8% with alcohol, 35.3% with bleach (20.7% boil, and 37.8% use other methods. The most frequent settings in which users injected were their own (63%) or friends' homes (17%). Only 4.3% infected in a shooting gallery. Males sharing needles in the past year (N=57) had more sexual artners (mdn = 2.0) than those not sharing needles (N=128, mdn = 1.0; *U*=2928, p<.02). Thus, youth, lack of time in treatment, cocaine use, and sexual promiscuity were all associated with higher rates of needle sharing. Nevertheless, the frequency of bleach use implies a specific concern about HIV transmission, and the current avoidance of needle sharing by most subjects (derived in part from their willingness to seek sterile injection equipment available in area drug stores) offers one explanation for the low HIV seroprevalence.

AFFILIATIONS: Veterans Affairs Medical Center and Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle

Genesis of New York City's Experimental Needle Exchange Program: Getting a Denigrated Group on the Government Agenda

Cherni L. Gillman

It is imperative to investigate what governmental agencies are doing in response to the AIDS epidemic among intravenous drug users (IVDUs). New York City took three years to approve a small experimental needle exchange program. This case study demonstrates how a private advocacy organization influenced gaining assent for this program in 1988.

Broad forces in New York City determine governmental response to an issue as controversial as a needle exchange program for addicts at risk for AIDS. This study identifies these forces and analyzes the policy formulation of the needle exchange program from a sociological perspective. It shows that when dominant political forces perpetuate a status quo and fail to respond to an epidemic, a private advocacy organization representing a competing ideology is 'needed to compel the government to act.

The study employs a tri-level framework of inquiry. It reviews the stark data of the wider context of AIDS among IVDUs: the federal Centers for Disease Control (CDC) estimate that at least 50% of New York City's 200,000 addicts test positive for HIV, human immunodeficiency virus, the virus that causes AIDS.

The arrangements of power that have culminated in a deadlock are discussed: our dominant legal and medical structural interests label illegal drug use as either a crime or a disease. Their priority is using punishment or treatment to fight action instead of formulating strategies to prevent AIDS. The issue has thus been turned into a choice between combatting addiction or AIDS.

This analysis characterizes ADAPT (the Association for Drug Abuse Prevention and Treatment) as a repressed structural interest engaged in agenda-building to protect the health needs of intravenous drug users. New York City's Department of Health is seen as a challenging structural interest because its mandate is prevention, not reforming human behavior (whether through treatment or punishment).

The concrete strategies engaged in by ADAPT to activate governmental response to the plight of intravenous drug addicts in the AIDS epidemic are discussed in careful detail. These include enacting the environment by taking a pro-active rather than a reactive stance; increasing the organization's political

activity; reaching for sources of support from those it serves; and incorporating symbolic elements to broaden its appeal.

The thesis of this paper is that a public health model is fighting to gain prominence as a competing ideology in order to challenge the status quo of health care for IVDUs in New York City. This competing ideology is necessarily represented by an external agent because governmental response by elected officials, health departments and law enforcement personnel is not easily achieved.

The research presented was conducted as a case study because the focal event (approval of the needle exchange program) occurred within a circumscribed area (New York City) and time (1988). The comprehensiveness that case study offers was another reason for choosing this method. Since the needle exchange is the city's only new project investigating how to formulate AIDS prevention efforts among IVDUs, it merits close examination. There are no analogous city programs with which to compare it. Additionally, case study has been used to investigate controversy and legislation surrounding other public health issues (such as child abuse, flouridation and smoking in public places) which are similar to analyzing governmental response to AIDs among IVDUs.

AFFILIATION:

Narcotic and Drug Research, Inc.
11 Beach Street, 3rd Floor
New York, N. Y. 10025

PLEASE ADDRESS ALL MAIL TO:

Dr. Cherni L. Gillman
771 West End Ave. #2A
New York, N. Y. 10025

Responses to I.V. L-Tryptophan in MDMA Users

L. H. Price, G. A. Rlcaurte, J. H. Krystal and G. R. Heninger

Methylenedioxymethamphetamine (MDMA or "Ecstasy") is a recreational drug and putative adjuct to psychotherapy that has proven to be a selective serotonin (5-HT) neurotoxin in laboratory animals. We assessed the long-term effects of MDMA use on 5-HT function by comparing the responses to the 5-HT precursor, tryptophan (TRYP) in MDMA users and matched healthy subjects.

METHODS: Nine MDMA users (7 male, 2 female; mean age of 34 ± 7(SD) years) and Nine Healthy subjects (7 male, 2 female, mean age of 33 ± 8 years) participated in testing. MDMA user total accumulative MDMA dose was 13.3 ± 13.4 g. They last used MDMA 66 ± 50 days prior to testing. After an overnight fast, TRYP, 7 g., was infused intravenously over 20 minutes.

Serum prolactin (PRL) and subjective mood ratings were obtained before and 90 minutes post-infusion. Between group data were analyzed using 2-tailed Wilcoxon Signed-Ranks Tests and within subject data were analyzed using 2-tailed Mann-Whitney U Tests.

RESULTS: In the control group, TRYP caused significant increases over baseline in the peak PRL response (11.0 ± 13.1 ng/ml, $p<.008$) and the area under the curve (AUC)

of the PRL response (568.8 ± 762.5 ng-min/ml, p<.02). In the MDMA group, peak PRL change (5.9 ± 8.5 ng-ml/min, p<.07) and the AUC PRL response (224.8 ± 491.9 ng-min/ml, p<.09) did not differ statistically from baseline or controls. Also, mood responses to TRYP did not differ between groups. Peak PRL TRYP response was 46% lower and AUC PRL response was 60% lower in MDMA users than controls.

COMMENT; In light of recent preclinical evidence that hypothalamic 5-HT systems may be relatively resistant to MDMA neurotoxicity, this study provides suggestive evidence of diminished 5-HT function in MDMA users.

AFFILIATIONS:

Yale University School of Medicine
New Haven, CT
and
Johns Hopkins University School of Medicine
Baltimore, Maryland

Human D-Amphetamine Drug Discrimination: Testing With D-Amphetamine and Hydromorphone

R. J. Lamb and J. E. Henningfield

Experimental studies have shown that a wide variety of drugs can serve as discriminative stimuli, and researchers have speculated that these discriminative stimulus effects are similar to the subjective effects measures used in many human studies. This study examined whether humans with histories of stimulant and opioid abuse would learn to discriminate the effects of d-amphetamine, and whether hydromorphone would then occasion amphetamine-approrpiate responding. Further, this study collected self-reports of the subjective effects of d-amphetamine and hydromorphone in order to examine to what extent the subjective effects of these two drugs co-vary with their discriminative effects.

METHODS

Five male subjects with histories of both stimulant and opioid abuse were trained to discriminate the presence and absence of d-amphetamine (30 mg po). Subjects responded under a second-order color-tracking procedure. Discrimination sessions were held 3-hours after drug administration. On their initial training session subjects were administered the training dose of d-amphetamine, and told this is drug A; when you receive drug A, you can earn extra money by responding on the green lever, when you do not get drug A, you should respond on the red lever to earn extra money. Subjects, also, completed a battery of subjective effects forms on a computer at 0.5, 1.0, 2.0, and 4.0 hours after drug administration. Subject vital signs were collected at these time-points as well. The subjective effects battery included the Single Dose Questionnaire, the MBG, LSD, PCAG, BG, and A scales of the Addiction Research Center Inventory (ARCI), and analog ratings of drug-liking, drug-produced good and bad effects, and drug strength.

RESULTS

Subjects readily acquired this discrimination. Administration of the training dose of d-amphetamine occasioned increases in self-reports of feeling the drug, reports of the drug being most like a stimulant, the MBG and A scales of the ARCI, reports of drug-liking, and decreases in reports of the drug being most like a blank.

After acquisition of the discrimination, test sessions with various doses of *d*-amphetamine and hydromorphone were held. Administration of d-amphetamine (3.75-45 mg, p.o.) produced dose-related increases in amphetamine appropriate responding. Administration of hydromorphone (1-12 mg, po) did not occasion d-amphetamine appropriate responding.

Administration of d-amphetamine produced dose-related increases in reports of drug-liking, the MBG and A scales of the ARCI, reports of feeling the drug, and decreases in reports of the drug being most like a blank.

Administration of hydromorphone produced dose-related increases in reports of drug-liking, the MBG and A scales of the ARCI, reports of feeling the drug, and decreases in reports of the drug being most like a blank. These effects were similar in magnitude to those produced by d-amphetamine.

Administration of d-amphetamine produced dose-related increases in reports of the drug being most like a stimulant, while hydromorphone produced dose-related increases in reports of the drug being most like dope. Conversely, administration of d-amphetamine did not produce dose-related increases in reports of the drug being most like dope, nor did hydromorphone produce increases in reports of the drug being most like a stimulant.

During test sessions, in 16 of 16 sessions in which responding was on the amphetamine-appropriate lever at least one report of the drug being most like a stimulant was made; conversely, in none of the 24 sessions that responding was not on the amphetamine-appropriate lever was there at least one report of the drug being most like a stimulant. In contrast, the discrimination performance did not similarly parallel other self-report measures, such as reports of drug-liking or responses on the MBG or A scales of the ARCI.

DISCUSSION

In this study subjects readily learned to discriminate the effects of the training dose of amphetamine. Administration of various doses of amphetamine led to a dose-related increase in amphetamine-appropriate responding. Increases in amphetamine-appropriate responding were accompanied by changes in self-report measures. Administration of various doses of hydromorphone did not occasion amphetamine-appropriate responding, despite that the administration of hydromorphone led to similar increases in most self-report measures as were seen after amphetamine administration. The one measure that clearly distinguished between hydromorphone and amphetamine were reports about which drug the administered drug was most similar to. These drug identification results were closely correlated with the drug discrimination results. In test session on which responding occurred on the amphetamine-appropriate lever, subjects always made at least one stimulant identification; In the 24 test sessions were subjects did not respond on the amphetamine-appropriate lever there were no sessions in which a subject made a stimulant identification.

These results indicate that subjects with history of stimulant and opioid abuse can learn to discriminate the effects of d-amphetamine, that this discrimination can be drug class specific, and that this discrimination behavior is highly correlated with reports of the drug being most like a stimulant.

AFFILIATION: University of Medicine & Dentistry, Camden, N. J.
National Institute on Drug Abuse, Addiction Res. Ctr., Baltimore, MD

Alcohol Effects on Plasma Estradiol Levels Following LHRH Administration to Women

Jack H. Mendelson, Nancy K. Mello, Siew Koon Teoh and James Ellingboe

Alcohol abuse and dependence may cause severe disorders of menstrual cycle function in women, including persistent amenorrhea, anovulation, luteal phase dysfunction and ovarian pathology. The purpose of this study was to determine the effects of alcohol on anterior pituitary and gonadal hormones following LHRH stimulation in normal women. Plasma levels of estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin and progesterone were measured in adult women prior to and following intravenous administration of 100 mcg luteinizing hormone releasing hormone (LHRH) and oral intake of 0.694 gm ethanol per kilogram of body weight or placebo. These studies were carried out during both the follicular (N=12) and luteal (N=12) phases of the menstrual cycle. Mean peak blood ethanol levels of 113 to 122 mg/dl were detected 45 to 60 min following initiation of alcohol intake. LHRH stimulated a significant increase in LH, FSH and prolactin during the follicular and luteal phases of the menstrual cycle ($P < 0.001$), but there were no significant differences in stimulated hormone levels between the alcohol and placebo conditions. Plasma E2 levels did not increase significantly after LHRH administration and placebo, but there was a marked increase in plasma E2 levels ($P < 0.0001$) after concurrent administration of LHRH and alcohol. The increase in plasma E2 after concurrent LHRH and alcohol administration exceeded baseline plasma E2 levels by 60%. The alcohol-induced stimulation of E2 following LHRH administration may be due to changes in steroid biotransformation by the liver as a consequence of alcohol's effects on intrahepatic metabolic processes. It is also possible that alcohol has a direct effect on ovarian steroidogenesis of E2 following administration of LHRH. We conclude that the alcohol-induced increase in E2 may contribute to suppression of folliculogenesis with resulting anovulation and luteal phase dysfunction.

ACKNOWLEDGEMENTS

This research was supported in part by Research Scientist Awards DA00064 and DA00101 from the National Institute on Drug Abuse and Grants AA06252 and AA04368 from the National Institute on Alcohol Abuse and Alcoholism.

AFFILIATION

Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02178

268-365 0 - 90 -15 : QL 3

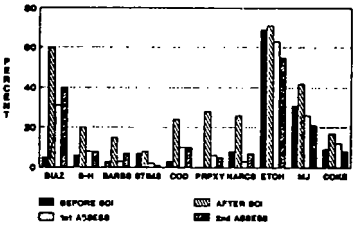
Substance Use and Receipt of Treatment in Persons with Recent Spinal Cord Injuries

Sidney H. Schnoll, Allen W. Helnemann, Matthew Doll and Kevin J. Armstrong

This study assessed the rate of self-reported substance use, consequent problems, perceived need for treatment, and receipt of treatment by persons with long-term spinal cord injury (SCI). Information was obtained from 86 persons with traumatic SCI who were between 13 and 58 years at injury, cognitively intact, injured more than one year, English speaking, and recruited from two SCI organizations. The mean age was 39.5 years at recruitment; and 69% were men. Participants were assessed at two time periods 1 year apart. They reported on drug use during four periods in their lives: 1) the time prior to their SCI (Before SCI); 2) the time after their SCI (titer SCI); 3) the 6 months prior to the 1st assessment (1st Assess) and 4) the period between the 1st and 2nd assessment (2nd Assess). The drugs reported on were diazepam (DIAZ), sedative-hypnotics including other benzodiazepines and non-barbiturate sleeping pills (S-H), barbiturates (BARBS), stimulants other than cocaine (STIMS), codeine containing analgesics (COD), propoxyphene containing analgesics (PRPXY), narcotic analgesics other than codeine and propoxyphene (NARCS) , ethanol (ETCH) , marijuana (MJ) , and cocaine (COKE). All of the participants reported use of substances with abuse potential at some time in their lives: the time of greatest use was during the immediate post-injury time period. Use was defined as use on three or more occasions.

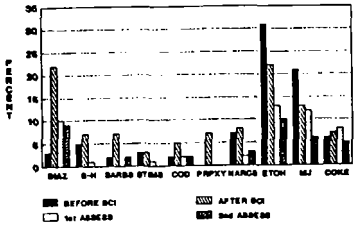
Figure 1 shows drug use patterns across all of the time periods. In all drug categories, use increased immediately after SCI with a reduction in use over time. Over 60% of the sample used alcohol prior to SCI with 55% still using at the time of the last assessment . Over 20% of the sample were still using marijuana at the time of the last assessment and cocaine use returned to pre-SCI use levels.

Figure 1



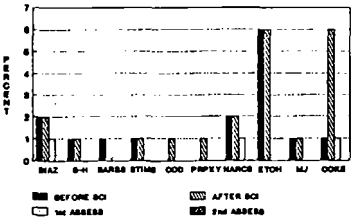
Problems related to drug use (Figure 2) were greatest after SCI except for alcohol and marijuana. With these two substances, problems were greatest before SCI. In all drug groups, the number of subjects reporting problems decreased over time. Of the prescription drugs, diazepam, the most frequently prescribed drug, created the most problems. Problems with the non-prescription drugs were higher than for the prescription drugs with cocaine sharing the largest number of problems in relation to the number of users.

Figure 2



Few of the subjects reporting problems reported needing help (Figure 3). Most often help for drug use was perceived either before SCI or after SCI. Of the subjects using cocaine after SCI, 1/3 reported needing help. Of those reporting need for help, very few actually received help: diazepam, 1% before and after SCI; sedative-hypnotics, none; barbiturates, none; stimulants, none; codeine, 1% before SCI; propoxyphene, 1% after SCI; narcotics, 2% before and after SCI, 1% at 1st assessment; alcohol, 1% after SCI; marijuana, 1% after SCI; and cocaine, 2% after SCI.

Figure 3



Participants who reported a need for treatment regarding substance abuse did not receive treatment for a variety of reasons. Twelve percent of the sample reported changing their mind, 8% did not receive treatment because they thought they could not change their use, 3% reported that they could not afford treatment, 3% reported they did not know where to get help, 3% reported that others said that treatment was not necessary, and 28% endorsed "other" as the reason they did not receive treatment and specified that they felt they could change themselves.

Abuse of both prescription and nonprescription drugs was reported, suggesting the importance of close monitoring of patients with histories of substance abuse and who are prescribed sedating, tranquilizing, or narcotic medications. The proportion of persons reporting substance use on 3 or more occasions varied from substance used and the time of the assessment. Further, 70% of the sample reported one or more problems related to substance use; 16% reported believing they needed treatment for their substance use while only 7% reported receiving treatment.

Affiliation: Medical College of Virginia/Virginia Commonwealth Univ. Richmond, VA, Illinois Institute of Technology, Chicago, IL., and Northwestern University Medical School, Chicago, IL

Effect of Dose on Nicotine's Withdrawal-Suppressing, Adverse and Discriminative Stimulus Effects in Humans

John R. Huges, Steven W. Gust, Robert M. Keenan and James W. Fenwick

Seventy-eight smokers were randomly assigned to 0, 0.5 mg (1 mg unbuffered), 2 mg and 4 mg doses of nicotine gum. Withdrawal, side effects and discrimination of active gum were measured via observer and self-report 1 week post-cessation. Higher doses did not more effectively suppress total self-reported or observed withdrawal (0.5=2.0=4.0>0.0). Impatience and irritability *were* more dose related than other withdrawal symptoms. Higher doses increased total side effects in a linear fashion (4.0>2.0>0.5>0.0) and especially increased vomiting and stomachache. Higher doses also did not induce more accurate discrimination of active vs placebo gum (0.5=2.0=4.0>0.0). The ability of low dose nicotine to relieve withdrawal and be classified as active gum could be due to overcoming a low threshold or to a combination of subtle peripheral effects plus instructional effect. Whatever the reason, our results suggest the shape of the dose-response curve varies across nicotine's withdrawal-suppression, adverse and discriminative stimulus effects.

AFFILIATION

Human Behavioral Pharmacology Laboratory, Departments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401

The Effects of Smoking Deprivation on Caloric Consumption in Women With Bulimia Nervosa

Cynthia M. Bulik, Ronald Dahl, Leonard H. Epstein and Walter Kaye

INTRODUCTION

It has been clearly established that smokers weigh less than comparable non-smokers and that cessation of smoking generally results in weight gain. The inverse relation between smoking and body weight is known to the general public and is an important motivational factor for smokers. Given preliminary survey findings of high rates of smoking in inpatient women with bulimia nervosa (75% in comparison to the 25-50% rate recorded in the young adult female population by the United States Public Health Service), we chose to further examine this relationship by studying the effects of smoking deprivation on eating behavior in women with bulimia nervosa who were regular smokers. We hypothesized that: 1) caloric consumption would increase under laboratory non-smoking conditions; 2) the effect of smoking on caloric intake would be dose dependent; and 3) urges to binge would increase under non-smoking conditions.

METHODS

Five women between the ages of 21-30 who met DSM III-R criteria for bulimia nervosa and who were regular smokers participated in a within subjects design. Each session lasted from 8 am-12 pm on four consecutive days. Subjects first had an habituation day and then were randomly assigned to order of presentation of regular smoking (RS), low nicotine (LN), and non-smoking (NS) conditions. Subjects were asked to abstain from both eating and smoking from 9 pm prior to each study day. Expired air COa measures were taken before and after each session.

The study was conducted in a research room equipped with vending machines from which. all food and cigarettes were dispensed and automatically recorded on a connected computer. Uneaten portions were subtracted from the original weight to determine amount consumed. Caloric content was determined using the United States Department of Agriculture Handbook 456.

On each of the experimental days, subjects ate ad lib. On the RS day, subjects smoked their own brand of cigarette each half hour on cue. On the LN day they smoked Carlton cigarettes (0.2 mg nicotine) with a 76% extraction filter (holder). On smoking days, puff duration and interval were prescribed. On the NS day subjects drew lines each half hour on cue. Subjects used cigarette holders on each day to control for consistency. Events were controlled by the audio portion of a videotape. There was no experimenter contact throughout the session.

RESULTS

All subjects complied with the non-smoking request (COa<13 ppm). The post-session COa measures were: RS (43.6 ppm; range 36.5-49.5); LN (20.6 ppm; range 14.0-24.5); and NS (9.06 ppm; range 5.5-15.5).

Data were analyzed using a repeated measures analysis of variance. Average caloric intake across the three experimental conditions was: RS (477.8 kcal ± 295.2), LN (509.1 kcal ± 206.5), and NS (832.0 kcal ± 405.8) ($F=5.07$, $p<.04$). Neuman-Keuls post-hoc significance tests indicted that the non-smoking condition was significantly different than both of the smoking conditions, but that the smoking conditions did not differ from each other. Two subjects approached binge levels of consumption (1116 and 1398 kcal) on the NS day.

Macronutrient composition of food eaten indicated that calories of fat consumed were significantly greater in the NS condition (208.3 kcal ± 148.1) than in the RS (110.2 kcal ± 107.8) or LN (115.4 kcal ± 72.0) conditions. Fat consumption did not differ between the LN and RS days. Calories of proteins and carbohydrates did not differ across conditions. There were no significant differences in self-reported urges to binge across the three experimental days.

CONCLUSIONS

In conclusion, this study demonstrated that caloric consumption increases under laboratory non-smoking conditions in women with bulimia nervosa who are regular smokers, and that smoking may serve to modulate food consumption in this population. Behavioral economic theory suggests that in their usual environment, both cigarettes and food are available as reinforcers to these individuals. When constraints are placed on access to one reinforcer (cigarettes), the value and hence rate of consumption of the alternative reinforcer (food) increases. Additional studies using more controlled methods of smoking self-administration as well as non-nicotine cigarettes will aid in the further determination of the effects of dose and the relative contribution of the behavioral and pharmacological aspects of smoking to the observed effect on caloric intake in women with bulimia.

University of Pittsburgh School of Medicine, Department of Psychiatry

Zolpidem and Triazolam in Humans: Behavioral Effects and Abuse Liability

Surette M. Evans and Roland R. Griffiths

Zolpidem, an imidazopyridine, is a novel non-benzodiazepine structurally, which binds preferentially to central omega$_1$ receptor subtypes. The pharmacological profile of zolpidem is in general similar to that of benzodiazepines although zolpidem appears to be preferentially active as a hypnotic and seems to lack some of the undesirable effects of benzodiazepines such as residual effects the next day and decrements in memory. At this time zolpidem is marketed in Europe as a hypnotic. The present study was undertaken to provide information about the behavioral effects and abuse potential of zolpidem by directly comparing zolpidem to the currently marketed benzodiazepine hypnotic, triazolam, in subjects with histories of sedative abuse.

In an outpatient setting the acute effects of placebo, zolpidem (15, 30 and 45 mg) and triazolam (0.25, 0.5 and 0.75 mg) were assessed using a double-blind, cross-over, randomized design. Subjects were 15 healthy male volunteers with histories of sedative and/or alcohol abuse. Drug effects were assessed with objective performance tasks, observer ratings of drug effect and subject ratings of strength of drug effect, mood, drug liking and monetary street value.

Both triazolam and zolpidem produced dose- and time-related effects on subjective and behavioral measures. The onset of drug effect was rapid for both drugs; usually by 30 min to 1 hr there were detectable effects. Analysis of time to peak drug effect for the 2 highest doses of triazolam and zolpidem showed no difference between the two drugs.

Both triazolam and zolpidem produced dose-related decrements in performance on a variety of psychomotor tasks including balance, circular lights, enter and recall, digit symbol substitution test and reaction time. On the picture recall/recognition task all doses of triazolam decreased the number of pictures recalled immediately after presentation whereas only the highest dose of zolpidem decreased the number of pictures recalled. On delayed recall, all doses of triazolam and zolpidem produced significant decreases. The analysis of variance comparing the two drugs showed that triazolam produced significantly more impairment than zolpidem on both immediate and delayed recall.

There was a dose-related increase in the percentage of subjects identifying triazolam as a sedative, up to a maximum of 87% at 0.75 mg triazolam. At the two lower doses, zolpidem was identified as a sedative to the same extent as

triazolam. However, at the highest dose of zolpidem only 33% of subjects identified the drug as a sedative.

Both drugs produced dose-related increases on observer-rated strength of drug effect. There were also dose-related increases in subject ratings of strength of drug effect and drug liking for both drugs. In contrast, zolpidem. but not triazolam, significantly increased subject ratings of bad effects, anxious/nervous, light-headed/dizzy and queasy/sick to stomach. In addition, there were 8 incidents of vomiting following zolpidem administration and none following triazolam administration. Overall, the results suggest that zolpidem and triazolam produce similar effects on objective performance measures, observer-rated measures and subject-rated measures such as strength of drug effect and drug liking. Unlike triazolam, however, zolpidem also produces a profile of adverse subjective effects which may limit the abuse potential of this drug. Supported in part by NIDA Grant DA-03889.

AFFILIATION

Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224

Can Buspirone Substitute for Benzodiazepines in all Anxious Patients?

Richard I. H. Wang, Domingo Tiuseco, Byung L. Roh, Jung-Ki Cho and Carol Kochar

In the search for antianxiety agents devoid of some of the undesirable effects of the benzodiazepines, the non-benzodiazepine, buspirone was investigated. Buspirone was shown to have tranquilizer activity. It has little or no sedative effect and it lacks anticonvulsant and muscle relaxant action. It does not interact with other CNS depressants including alcohol. It does interact with dopamine receptors in the brain and is believed to have dopamine agonist as well as antagonist activity. Buspirone produces no effect on the GABA system and does not bind to the benzodiazepine receptor.

Because of the lack of abuse potential plus its antianxiety activity without sedation, buspirone appeared to be an ideal compound for 3 groups of our patients: 1) patients with generalized anxiety disorders on the methadone maintenance Program, 2) patients who exhibit a great deal of anxiety and craving for alcohol following the short period of acute alcohol detoxification, and 3) patients with severe anxiety of a chronic nature. In the first group of patients using various methods including psycho-supportive therapy, we found the methadone maintenance patients frequently used benzodiazepines which was confirmed by urine surveillance. Upon confronting the patients, we learned that they were taking diazepam, alprazolam, or lorazepam, which they obtained from other physicians inside and outside of our hospital and/or illegally through friends. In order to reverse this noncompliance to treatment plans, six of these patients on methadone maintenance participated in our study. All were male patients. They were given buspirone 10 mg TID for treatment of their benzcdiazepine dependence. Urine surveillance was done twice per week. Anxiety rating scales were completed before starting treatment with buspirone and weekly thereafter. It was found that on buspirone 10 mg TID, the anxiety score remained high and unchanged. The urine continued to show positive for benzcdiazepines. These patients unanimously demanded to discontinue the buspirone medication.

In our second group of post-alcohol detoxification patients, it was expected that buspirone would have tremendous value in the treatment of continued symptoms of anxiety, nervousness and craving for alcohol. Eight patients who continued to have this symptomatology participated in the buspirone study. The same urine surveillance and rating forms were done before starting

buspirone and weekly thereafter. Psycho-supportive therapy continued as usual. At the end of one and two weeks of treatment with buspirone, it was found that there was no imporvement of symptamatology. Because of this, buspirone medication was discontinued and patients were placed on low doses of benzodiazepines.

The third group of patients with chronic severe anxiety received buspirone 10 mg QID for two weeks. All three patients continued to experience severe nervousness. Cur lack of effectiveness of buspirone in these three groups of patients was discouraging. It indicated to us that a potentially non-dependent producing antianxiety drug may not be available for our types of patients.

Looking into the existing literature on the clinical efficacy of buspirone, one finds two types of therapeutic responses. One group of workers found buspirone to be equivalent to diazepam, chlorazepate, alprazolam or lorazepam. The other group of workers found buspirone to be ineffective in relieving anxiety, particularly on any patient previously taking benzodiazepines. A possible explanation for the effectiveness of buspirone is the fact that a large percentage of patients in the study were female. Some important points were not taken into amount, such as, there was a week of placebo washout before the study. This is a condition usually unsuitable for patients with reasonable degrees of severity in anxiety to stay on the study. There was also sizable dropout, not only among placebo patients but also on the active medications.

It should be pointed out that it is unusual for the physicians to tell their patients not to expect an overnight success after taking buspirone, and it is impractical to forewarn the newer patients that a maximal antianxiety effect may take 2 to 3 weeks. Only the mildly anxious patients can withstand the lengthy delay for the onset of therapeutic action. In summary, we have studied 3 types of patients with generalized anxiety disorder requiring anxiolytic therapy. All three groups of patients, whether they were on methadone maintenance program, post-acute alcohol detoxification or chronically severe anxiety patients, failed to respond to the carefully and strenuous treatment with buspirone 10 mg TID to 10 mg QID for 2-3 weeks. Clinics are confronted with the problem of misuse and abuse of benzodiazepines. In view of the controversy of the existing findings on the efficacy of buspirone, better controlled studies are needed to assess the role of buspirone in the treatment of anxiety in a variety of patients.

REFERENCES furnished upon request.

AFFILIATION: V.A. Med. Ctr., Med. Col. of WI., Milwaukee, WI 53295

Human Aggressive and Non-Aggressive Responding During Acute Tobacco Abstinence

D. R. Cherek, R. H. Bennett, J. D. Roache and J. Grabowski

Four male tobacco smoking subjects have participated in a study to determine if changes in human operant behavior can be detected during brief eight hour exposure to tobacco abstinence. Subjects remained in the experimental area from 8:00 AM to 4:30 PM five days per week. Each day subjects participated in five sessions (25 min duration), beginning at 9:00AM, 1O:OOAM, 12:00 PM, 2:OOPM and 4:OOPM. At 8:30 AM each day: (1) an initial carbon monoxide (CO) reading was taken, (2) an expired air sample was taken for alcohol level determination and (3) a urine sample was taken for drug screen analysis. Alcohol and drug free samples were required. If the initial morning CO value was below 21 ppm, subjects were paid five dollars.

During the sessions, subjects could respond on lever A or B. Lever A responding was maintained by a fixed-ratio (FR) 100 schedule of point-presentation (1 point = ten cents). Lever B responding ostensibly subtracted points from a fictitious person on an FR 10 schedule and was defined as aggressive. Aggressive responding was engendered by subtracting points from the subjects and maintained by initiation of 250 sec intervals free of point subtractions. Point subtractions were attributed to the other people participating in the experiment.

During the time (approx. 6 hrs) subjects were not in sessions, they were exposed to four different experimental conditions. Under baseline conditions, ad libitum smoking of their preferred brand of cigarettes was permitted. At other times subjects were exposed to three tobacco abstinence conditions during which smoking was not permitted during the 8.5 hr experimental day: (1) nicotine gum, (2) placebo gum and (3) no gum. Under nicotine and placebo gum conditions, subjects were given two pieces of 2 mg nicotine or placebo gum to chew thirty minutes before each session. Baseline conditions were in effect until operant responding stabilized over 10-15 sessions. Subjects were then exposed to one of the three tobacco abstinence conditions for an entire day, and then returned to baseline conditions. These

alternating conditions (baseline vs abstinence) continued until subjects were exposed to each tobacco abstinence condition twice.

All four subjects reported smoking thirty to fifty high nicotine delivery cigarettes per day for ten or more years. Data collected in the laboratory also indicated that subjects were nicotine dependent: (1) subjects typically smoked about 20 cigarettes during the six hours of free time, (2) a blood sample taken during participation indicated high nicotine intake with cotinine values between 300-660 ng/ml, and (3) their answers to the Fagestrom Questionnaire indicated a high degree of nicotine/tobacco dependence (x=9.5).

Carbon monoxide (CO) measurements were taken at the end of each session. These measurements indicated that subjects were smoking throughout the day during baseline conditions, and subjects were not smoking during abstinence conditions.

Compared to baseline ad libitum smoking conditions, all four subjects had large increases in aggressive responding under the no gum condition. Aggressive responding was increased somewhat under placebo gum conditions, while under nicotine gum conditions aggressive responding was unchanged.

Non-aggressive (responses maintained by point presentation) remained essentially unchanged under smoking and abstinence conditions.

Our results indicate that under laboratory conditions increases in aggressive responding are observed when dependent tobacco smokers are exposed to an eight hour period of tobacco abstinence. These results are consistent with subjective reports and clinical observations of increased irritability during tobacco withdrawal.

This research was supported by NIDA grant DA 04044.

Substance Abuse Research Center
Department of Psychiatry and Behavioral Sciences
University of Texas Health Science Center at Houston

Physical Dependence on and Toxicity From Caffeine

John R. Hughes, Stephen T. Higgins, Warren K. Bickel, William K. Hunt, and Sara Pepper

In Study 1, ten moderate coffee users underwent 4 - 6 independent, randomized, double-blind, cross-over trials of one day of 4 cups of decaffeinated coffee and one day of 4 cups of decaffeinated coffee + 100 mg of caffeine. This trial was immediately followed by a test for self-administration of caffeinated vs. decaffeinated coffees. Study 2 was a direct replication using twelve subjects. The results were similar across studies.

Headaches, drowsiness, and fatigue were significantly greater on the decaffeinated coffee days than on caffeinated coffee days and occurred repeatedly in several subjects. Headaches, drowsiness and fatigue were prevalent (40 - 60% of trials) and were rated severe in a substantial minority of trials (10% to 15%). The *occurrence* of headaches, drowsiness, and fatigue on the decaffeinated days predicted subsequent self-administration of caffeinated coffees.

Anxiety, diuresis, dizziness, muscle twitches, tinnitus, sweating, talkativeness and tremulousness were greater on caffeinated coffee than on decaffeinated coffee. These symptoms were less reliable and prevalent and of a smaller magnitude than the withdrawal symptoms. In addition, they did not predict avoidance of self-administration of caffeinated coffee.

AFFILIATION

Human Behavioral Pharmacology Laboratory, Departments of Psychiatry and Psychology, University of Vermont, Burlington, VT 05401

Effects of Controlled Nicotine Doses Upon Punished and Non-Punished Responding in Humans

R. H. Bennett, D. R. Cherek, J. D. Roache and J. E. Rose

The study reported here investigated the effects of the inhalation of tobacco smoke of varying nicotine content upon punished and non-punished operant responding in humans. The tobacco smoke was administered by the spirometry method which controls puff volume and ensures introduction of the smoke deep into the lungs.

Five male smokers (ages 23 - 37) participated in the study. Subjects' drug and alcohol use was monitored during the course of the study by urine analysis and daily breath alcohol measurements.

Subjects responded on a Lindlsey manipulandum to obtain points which accumulated on a digital counter. White and red stimulus lights were mounted to the left of the counter. All experimental conditions and data collection were controlled by a micro computer located in an adjacent room.

The spirometry method of tobacco smoke administration was utilized to administer a constant puff volume of the smoke deep into the lungs. This is a self contained system into which air is initially pumped into a 1 liter bag which is then clamped off. A syringe is used to draw 60 cc of smoke from a cigarette and injected into the system. The subject then inhales the smoke and air through a mouthpiece once the system is opened. The bolus of air behind the smoke forces the smoke deep into the lungs. One 60 cc puff of smoke is administered every 30 seconds during each administration period. Twenty puffs (10 from each of 2 cigarettes) are delivered at each administration period.

Lever pressing was maintained by a random interval 20 sec (RI 20) schedule of point presentation (1 point = ten cents). A punishment contingency which stipulated that points were subtracted on a variable ratio 30 (VR30) schedule, was also in effect. In the second half of the experiment, the punishment contingency was removed.

An experimental session consisted of two 20 minute components (pre and post smoke administration) separated by a smoke administration period. The subject's heart rate, blood pressure, and carbon monoxide expired air level were measured before and after each smoke administration. Subjects participated in 4 sessions daily (M-F).

On baseline session days subjects received 0.3 mg nicotine yield cigarettes during the smoke administrations. Experimental days were separated by baseline days and an experimental day was instituted only once baseline responding was stable. The three experimental manipulations were 1.2 and 2.7 mg nicotine yield cigarettes, and a sham procedure in which no smoke was delivered through the apparatus. Subjects responding was punished during the first part of the experiment and, once all three experimental manipulations were experienced, the punishment contingency was removed and experimental manipulations were administered a second time.

Similar CO boosts were observed with each of the different nicotine yield cigarettes. Mean heart rate changes (before and after smoke administration) increased with increases in nicotine yield of the cigarette indicating an effect of nicotine.

Mean change (before and after smoke administration) in punished responding showed a dose-related decrease as a function of nicotine yield. The sham procedure produced a mean increase of 23 responses while the 1.2 and 2.7 mg nicotine yield cigarettes produced mean decreases of 30 and 48 responses respectively. Three of the five subjects experienced the non-punished RI schedule. Two subjects showed nicotine-induced increases and one subject showed decreases in non-punished responding.

The CO boost and heart rate data showed that the spirometry method of smoke administration was effective in administering tobacco smoke in a controlled manner. The similar CO boosts across nicotine yield cigarettes demonstrates a controlled smoke delivery while a dose dependent increase in heart rate demonstrates nicotine yield (dose) control. The nicotine dose-related decrease in punished responding was similar to previous observations with CNS stimulants. The data for the non-punished responding was variable which may be related to very different baseline response rates of the individual subjects.

This research was supported by NIDA grant DA 04044.

Substance Abuse Research Center
Department of Psychiatry and Behavioral Sciences
University of Texas Health Science Center at Houston

Effects of Delta-9-THC on Repeated Acquisition and Performance of Response Chains in Humans

Warren K. Bickel, John Ft. Hughes and Stephen T. Higgins

Overall, generality of drug effects has been demonstrated between humans and non-humans responding on the repeated acquisition and performance of response chains. In the non-human laboratory, AS-THC has been demonstrated to produce no effects on monkeys and pigeons performing on the repeated acquisition procedure. The purpose of this study was to examine the effects of AS-THC on the repeated acquisition procedure in humans.

METHODS

Subjects: Four healthy adult volunteers (3 females, 1 male) between the ages of 21 and 32 served as subjects. Subjects had prior experience with marijuana.

Procedure: The repeated acquisition and performance of behavioral chains procedures was employed. The acquisition component required subjects to learn a lo-response sequence using three keys of a numeric keypad. The performance component required subjects to emit an already learned 10-response sequence on those same three keys. The keys had to be depressed in a predetermined order in the presence of the numbers 0-9, which appeared sequentially in the center of a video screen. Correct responses resulted in the subject moving to the next link in the chains. Incorrect responses initiated a 2-sec time-out, during which responding had no programmed consequences. Subjects also completed Visual-Analog rating scales of drug effects. Drug effects were assessed immediately predrug and postdrug and 30 minutes thereafter for 4 hours.

Drua Administration: Δ9-THC was administered orally in two opaque capsules. Doses were placebo, 10 and 20 mg. Subjects were exposed to the doses in a randomized order. Generally each dose was replicated three times.

RESULTS

Data from peak behavioral effect (4 hrs post-drug administration) were analyzed. In the acquisition component, overall percent errors generally increased under drug in three subjects. In the performance component, overall percent errors was generally unaffected by the drug. The selective effects observed between acquisition and performance is reflected in a cumulative plots of errors. The subject's rating of "strength of drug effect" and "good" effects were also increased by drug.

CONCLUSION

1. There were individual differences in the effects of Δ9-THC on percent errors on the repeated acquisition of behavioral chains. In three subjects, Δ9-THC produced increases in the acquisition component, while no effect of dose was observed in one other subject. This data may suggest that humans may be more sensitive to the disrupting effects of Δ9-THC than nonhumans

2. Doses of Δ9-THC that increased percent errors in the acquisition component had no effect in performance; that is, selective drug effects were obtained.

3. Drug produced changes self-report measures in all subjects. Thus, the subject showing no increase in acquisition errors received a behaviorally active dose.

ACKNOWLEDGEMENTS

Supported by PHS Grant DA-05538

AFFILATION

Human Behavioral Pharmacology Laboratory, Department of Psychiatry, University of Vermont, 1 South Prospect, Burlington, VT 05401.

Methadone Maintenance: High Rate of Other Substance Use Disorders and Relationship to Psychiatric Comorbidity

Robert K. Brooner, George E. Bigelow and Michael W. Regier

Opioid dependent, methadone maintained patients are known to have high rates of non-opioid substance use. However, less is known about diagnostic prevalence for specific classes of psychoactive substances. The present study determined the prevalence of DSM-IIIR diagnoses of Abuse or Dependence for a complete range of substances, and analyzed the prevalence of multiple substance-use diagnoses as a function of concurrent (non-substance) psychiatric diagnoses.

Participants were 68 methadone maintained subjects enrolled in an HIV testing and education program. All diagnoses were made using a structured interview.

Lifetime rates of Abuse or Dependence were 55.9% for Cocaine, 53% for Sedative/Hypnotics, 47.1% for Marijuana, 47.1% for Alcohol, 8.8% for Hallucinogens, 7.3% for other Stimulants and 1.5% for Inhalants. Forty-eight percent of the sample was positive for a non-substance use Lifetime psychiatric disorder, primarily involving Antisocial Personality Disorder (29%) and Major Depression (19%). A one-factor ANOVA revealed significant differences in the mean number of non-opioid substance use disorders for subjects with versus without any non-substance use psychiatric diagnosis ($p=.008$). A greater number of substance use diagnoses was found for subjects with versus without psychiatric diagnoses ($p<.05$). Some of the known problems in treating substance abusers with other psychiatric disorders may result from these patients having a more severe substance use clinical course.

AFFILIATION: The Johns Hopkins University School of Medicine
Baltimore, MD

Human Immunodeficiency Virus and Viral Hepatitis Seroepidemiology in New York City Intravenous Drug Abusers (IVDAs)

L. S. Brown, Jr., M. J. Kreek, C. Trepo, A. Chu, S. E. Banks, M. Valdes, D. Ajuluchukwu, R. Phillips and B. j. Primm

INTRODUCTION

Both human immunodeficiency virus (HIV) and hepatitis virus infections are prevalent medical disorders among intravenous (IV) drug abusers. The offending viral agents commonly associated with hepatitis among IV drug abusers include non-A/non-B hepatitis virus, hepatitis B virus (HBV), and hepatitis D virus (HDV). Previous investigations have raised the question of the efficacy of HBV immunity, conferred either through vaccination or a previous history of HBV infection, in the presence of HIV infection. Other researchers have shown that the pathogenesis of HDV infection is linked to HBV antigenemia. Still other questions remain as to the prevalence of concomitant HBV, HIV, and HDV infection even in a high risk group such as IV drug abusers. As a pilot to a larger HIV seroprevalence study, we report the results of HIV and hepatitis serology in association with behavioral information.

PATIENTS and METHODS

In October, 1987, 50 patients, enrolled in selected drug treatment clinics in New York City volunteered to participate in a human subject approved protocol. Twenty-eight percent of the subjects were female and 72% were male. The ethnic/racial composition included 40% black, 50% Hispanic, and 10% non-Hispanic white subjects. The ages ranged from 23-54 years with a mean of 34.4±1.0 (SEM) years. Duration of drug use ranged from 1-35 years with a mean of 15.8±1.2 years. Drug treatment enrollment ranged from 1-180 months with a mean of 35.9±6.6 months.

Trained interviewers administered a standardized questionnaire of demographic factors, drug abuse patterns and sexual behaviors, and medical information. Drug abuse patterns included type of drugs, frequency, and duration of drug use. Type of sex acts, frequency of sex, and number of sex partners were among the sexual behaviors examined. Sera was collected and analyzed for HIV-l antibodies using an enzyme-linked immunoassay (ELISA

from Abbott Labs). A Western blot assay (Genetic Systems) was used to confirm repeatedly positive ELISAs. Sera with p24 and/or gp41 bands on Western blot were considered positive. A highly sensitive monoclonal antibody assay and a standard radio-immunoassay, respectively, were used to detect the presence of hepatitis B surface antigen (HBsAg) and hepatitis core antibody (anti-HBc). Two different methods were used to assay for HDV serological markers. For delta antigen, a commerical kit (Noctech) was used with validation by one of us (CT). We used a commercial kit (Abbott) and an assay developed and validated by one of us to determine the presence of delta antibody. Using SPSS-PC+, univariate analyses was used to examine associations between positive HBV and HIV serology and other variables.

RESULTS

The HIV-l infection rate was 54% and 86% of the subjects had at least one marker for HBV infection. Serological evidence of anti-HBc, anti-HBs, anti-HBe, HBsAg, and HBeAg was found in 86%. 70%. 38%. 2%. and OX, respectively of the sample. No subject had serological evidence of HDV infection. Of the 6, 5, and 46 subjects, respectively, who admitted to sex for money, sex for drugs, and exclusive heterosexual behaviors, 50%. 60%, and 56.51, respectively, were HIV-l seropositive, while 83.3%. 80%, and 84.81, respectively, had at lease one HBV serological marker. For every sexual or drug abuse-related behaviors, HBV infection rates were higher than HIV-l prevalence rates. HIV-l infection was significantly associated with an older age (p=.011), a longer duration of drug use (p=.000), and anti-HBc (p=.007) serostatus. Serological evidence of at least one marker of HBV infection was significantly associated with a higher mean age (p=.002), a longer duration of drug use (p=.005), and a lower rate of participation in anal intercourse (p=.003).

CONCLUSIONS

The rates of HIV and HBV infection in this population were considerable in this population of patients enrolled in drug treatment. Because of the small sample size, results of this study must be interpreted with caution. Relatively long durations of drug treatment enrollment among these subjects may explain the low HDV infection rate. Two percent of patients with only HBV antigenemia vere at risk for HDV. These findings are consistent with similar mechanisms of transmission between HIV-l and HBV. Most importantly, 14% of this population had no markers of HBV infection and could benefit from HBV immunization. To the extent that this finding is widespread, HBV vaccination among drug treatment enrollees has significant public health benefits.

AFFILIATIONS: Division of Research and Medical Affairs, Addiction Research and Treatment Corporation, Brooklyn, NY; Department of Medicine. Harlem Hospital, College of Physicians and Surgeons, Columbia University, New York, NY; Rockefellar University, New York, NY; and Faculte Alexis Carrell, Lyons, France.

Stress and Emotional Distress as Possible Co-Factors in the Development of AIDS in a Sample of Intravenous Drug Users

Veronica Catan

Intravenous drug users have been identified as a group at risk for AIDS. It is important to identify co-factors which may compromise immune function and determine host response in this group.

Stress and emotional distress were chosen as co-factors to examine for two reasons: 1. Stressful life events occur more commonly among disadvantaged populations (Mechanic, 1986), and 2. Stress and emotional distress have been identified as factors in illness development.

The Sample

The sample consists of 147 intravenous drug users recruited at a methadone maintenance program in New York City. They are part of a study on risk factors conducted by Narcotic and Drug Research, Inc.

At entry into the study, none of the subjects had sought treatment for AIDS or AIDS related complex.

METHODS

A face to face interview using a structured questionnaire was administered in private. Information was gathered including medical history, drug using behavior including injection frequency, sexual behavior, emotional distress and mood, and frequency of occurrence of a number of stressful events. A follow-up interview was conducted nine months later. Twenty five ml of blood were collected from each subject at intake and at follow-up. HIV status and measures such as total lymphocytes, T4 cells, T8 cells, B cells, IGA, IGM, IGG and T4/T8 ratio were assessed.

Stressors which were assessed included chronic stressors such as homelessness and hunger (missing more than 9 meals per week), and acute events, such as losing a job, being arrested, and having a breakup of a close personal relationship. Stress was measured for events during the year preceding the first interview and during the nine months between time 1 and time 2. Distress was measured by anxiety and depression symptoms over the 30 days preceding the follow-up interview. Each distress item was scored between 0 and 5 depending on the number of days reported. A variable was computed, noncope, to look at equal weights of stress and distress combined.

RESULTS

Clinical AIDS is preceded by severe depletion of CD4 cells. Therefore, decline in CD4 cells has been used as a measure of HIV-related immunosuppression and was used in this study. Neither stress nor distress was significantly associated with decline in CD4 cell count for time 1 to time 2.

T-Tests comparing groups at time 2 who had less than 3 ARC symptoms (Group 1) and those who had more than 3 symptoms (Group 2) showed o significant differences in means for stress. On distress, Group 1 had a mean of 11.4 and Group 2 had a mean of 26.2 ($p = .029$). A computed variable giving stress and distress equal weight called noncope had a mean of 26.6 for Group 1 and 46.2 for Group 2 ($p = .003$).

DISCUSSION

This research suggests that stressful events alone are not associated with immunosuppression, but that stress combined with emotional distress may be associated with illness development. There are conflicting models concerning whether being ill is associated with distress or whether distress has some effect n illness development. Further analysis is being done which may clarify this issue.

AFFILIATION

Narcotic and Drug Research, Inc., 11 Beach Street, New York, NY 10013

Intravenous Heroin Use: Its Association with HIV Infection in Patients in Methadone Treatment

A. Chu, L. S. Brown, S. Banks, T. Nemoto and B. J. Primm

INTRODUCTION

Intravenous (IV) drug use is closely associated with human immunodeficiency virus (HIV) infection. The number of HIV infected IV drug users continues to rise. It is well documented that the sharing contaminated needles is an important mode of HIV transmission, but little is known about the relationship of other cofactors in HIV infection. This study examined the seroprevalence of HIV infection among IV drug users who were enrolled in methadone maintenance program, and more specifically the role of heroin use as a main factor in HIV infection.

METHODS

In 1987, 218 patients were recruited from methadone treatment clinics in New York City. A standardized questionnaire asking the patterns of drug use and demographics was administered by trained interviewers and sera was collected and tested for HIV antibody using enzyme linked immunosorbent assay (ELISA) and a confirmatory Western Blot.

RESULTS

The HIV seropositivity rate was 60.1%. Those who were HIV seropositive reported significantly higher frequencies of intravenous heroin use in the previous 12 months than those who were seronegative ($p=0.026$). Intravenous heroin users were more likely to be infected with HIV than non-IV users ($p=0.040$). Serostatus was clearly associated with the age of first intravenous drug use. The participants who started using heroin intravenously and were addicted at an early age were significantly more likely to be HIV seropositive. Whites participants first started using any drugs at a significantly younger age than blacks or Hispanics ($p=0.0466$). Whites also reported injecting heroin at a significantly younger age than blacks or Hispanics ($p=0.057$). The study also indicated that needle sharing behaviors were significantly associated with HIV positive status.

CONCLUSIONS

The IV heroin use -and an early age to start using heroin intravenously were strong predictors of HIV seropositive status. among LVDUs. More efficient education especilly focusing on younger age groups, is needed to stop AIDS epidemic among IVDUs.

AFFILIATIONS: Addiction Research and Treatment Corporation, Brooklyn, NY and Harlem Hospital, College of Physicians and Surgeons and Health Administration Division, School of Public Health, Columbia University, New York, NY.

Time Course of Detection of 6-Acetylmorphine in Urine After Heroin Administration

E. J. Cone and P. Welch

The confirmed presence of the heroin metabolite, 6-acetylmorphine (6-AM), in urine provides convincing evidence that heroin exposure occurred in the recent past. Presently, tests for detection of heroin use are based on detection of morphine, although it is recognized that sources of morphine in urine include heroin, morphine, codeine and poppy seeds.

We determined the time course of 6-AM and morphine in urine of 6 subjects following administration of single doses (3 and 6 mg, intramuscular) of heroin HCl. All subjects had a history of heroin abuse but were drug-free at the time of the study. Individual urine specimens were collected during the first 12-hr period after drug; thersafter, each subject's urine was collected as 12-hr pools. Specimens were frozen until analyzed by GC/MS. The appearances of 6-AM and morphine in urine were immediate. Levels of 6-AM declined rapidly and were not detectable after 8 hr, whereas morphine (total) was detectable for 18-24 hr. Overall, it appears that there is a very limited time window for detection of 6-AM after heroin use.

Authors Affiliations: E.J. Cone and P. Welch, Addiction Research Center, NIDA, Baltimore, MD 21224; J. Mitchell and B. Paul, Navy Drug Screening Laboratory, NAS, Norfolk, VA 23511

The Effects of 0.8 G.KG Ethanol on Cerebral Metabolism and Mood in Normal Volunteers

Harriet de Wit, John Metz and Malcolm Cooper

This study used positron emission tomography (PET) to evaluate the effects of a moderate dose of ethanol (ETH; 0.8 g/kg) on cerebral glucose metabolism (GM) in normal volunteers. The study explored the relationship between ETH-induced changes in CM and ETH-induced changes in mood.

Eight male, light social drinkers (average 5 drinks/week, aged 21-29) received two PET scans, during which they received either ETH (0.8g/kg) or placebo. Subjects first consumed a beverage and then were positioned in the scanner and injected with 6-8 mCi 18-FDG. During a 45-min period of FDG uptake, they performed a visual monitoring task (button-pressing in response to a light). The subjects' moods were assessed using the Profile of Mood States (POMS) prior to, and 20 and 40 min following beverage ingestion. Regional CM was determined using Sokoloffs model with standard rate constants and 14 regions of interest obtained from tomographic metabolic images.

In 7 out of 8 subjects, ETH decreased whole brain CM (means: placebo 8.88 mg/100g/min and ETH 8.40 mg/100g/min). None of the regions of interest were affected differentially by the drug. All subjects reported liking ETH more than placebo, and 6 out of 8 correctly identified the drug as ETH. The drug did not impair performance on the visual monitoring task but it did significantly increase measures of positive mood (e.g. POMS scales Vigor, Friendliness, Elation). Regional changes in CM were not correlated with relative changes in mood after ETH.

This dose of ETH had more consistent effects on CM and mood than a previously-tested, lower dose of ETH (0.5 g/kg): Most subjects exhibited decreased global CM, and all subjects reported increases in positive mood. Perhaps because of the relative homogeneity of responses to ETH, no relationships were found between regional CM and mood.

This research was supported by DE-AC02-86ER60438, the Alcoholic Beverage Medical Research Foundation and DA02812.

Department of Psychiatry, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637

A Dose Run-Up and Safety Evaluation of Nalmefene HCL in Human Volunteers

Paul J. Fudala, Rolley E. Johnson, Stephen J. Heishman, Edward J. Cone and Jack E. Henningfield

Four non-dependent, male volunteers (25 to 37 years of age) with histories of opioid abuse participated in a dose run-up/safety assessment of oral nalmefene HCl, 25-500 mg. Subjects received two capsules (containing nalmefene HCl or placebo) by mouth and an intramuscular injection (containing morphine sulfate, 15 mg or placebo) at intervals of at least 72 hours between doses. Morphine sulfate was always administered as the first treatment condition to ensure that subjects could discriminate opioid-like effects. One subject discontinued his participation for non-study related reasons after receiving nalmefene 25 mg and one discontinued participation after receiving 150 mg due to self-reported dysphoric effects. The highest doses tested in the two remaining subjects were 300 and 500 mg, respectively.

Since this was a non-randomized evaluation, no statistical analyses were performed on the data. While it appeared that nalmefene, at the doses tested, did not produce pupillary constriction or changes in any of the other physiologic parameters assessed (blood pressure, heart and respiratory rate, and oral temperature) morphine apparently produced pupillary constriction. Nalmefene produced elevations on LSD (dysphoria) and PCAG (apathetic sedation) scale scores in three of the subjects: morphine produced elevations of these scores in one subject. Only morphine produced apparent elevations on MBG (euphoria) scale scores (three subjects). Of the 15 times that nalmefene was administered, it was identified by subjects as an opiate on only 2 occasions while morphine was always identified by subjects as an opiate. Specifically, subjects identified nalmefene as blank (placebo), opiate, marijuana, Valium, downer, LSD, Thorazine, PCP, and "unknown, strange."

Subject-reported side effects (from a 10-item checklist) following nalmefene administration included "tiredness and sleepiness," "lightheadedness," and "dizziness." Subjects also volunteered reports (not part of the structured checklist) of "aching legs" and "unwanted erections." One subject reported that he felt violent and "strange" after receiving nalmefene,

75 mg, while another reported that he "felt as if he could fly" following 300 mg of the drug. No such descriptions followed the administration of morphine or have been reported following the administration of nalmefene in healthy volunteers in other studies.

Data obtained from this evaluation will be used to assess the physiologic and behavioral effects of nalmefene HCl in a double-blind, crossover study, and also to determine the appropriate dosages of nalmefene to be used in an assessment of its opioid-blocking activity.

AFFILIATION: NIDA Addiction Research Center, Baltimore, MD.

Comparison of the Behavioral Effects and Abuse Liability of Ethanol and Pentobarbital in Recreational Sedative Abusers

John J. Guarino, John D. Roache, William T. Kirk and Roland R. Griffiths

The similarities in the behavioral and psychomotor performance effect profile of ethanol and other sedative-hypnotics have long been recognized. Lacking, however, is a clear understanding of the relative abuse liability of ethanol to the other sedative-hypnotics. In previous studies from this laboratory, the dose-related behavioral, subjective and psychomotor performance impairing effects and the abuse liability of a number of drugs have been compared to standard drugs with known abuse liability. The purpose of this study was to investigate the dose-related effects of ethanol for a number of behavioral, psychomotor and subjective measures and particularly, for measures of abuse liability, and to compare the results with those obtained with a prototypic barbiturate, pentobarbital, under identical laboratory conditions.

Subjects were 8 healthy males with histories of alcohol and/or sedative abuse. Subjects resided on an 8-bed residential research ward throughout the study. A period of 3 to 7 days on the research ward were allowed for acclimatization to the tasks and the environment before dosing began. Subjects received doses of ethanol (0.5, 1 and 2 gm/kg or pentobarbital (150, 300, 600 and 750 ng/70 kg) in a within-subject double-blind, double-dummy Latin square design. Each day subjects received both a powdered fruit drink mix containing placebo or pentobarbital and orange juice with or without ethanol. On any given day placebo or an active dose of either ethanol or pentobarbital was administered. Each day, repeated measures were made of ethanol blood levels (BAL), objective performance (e.g., DSST, circular lights), subject and observer ratings of drug effect, and subject ratings of drug liking, mood and subjective drug effects. Data were analyzed as either raw scores, differences from baseline or percent of predrug performance.

The peak effect for most measures was between 1.5 and 3.5 hours after dosing for both pentobarbital and ethanol; higher doses required longer to attain peak effect and effects of these higher doses persisted longer. BAL recordings were orderly with respect dose and time and were consistent with the observed perfomance impairment to alcohol. Pentobarbital

and ethanol produced similar dose-related decrements in task performance on DSST, circular lights, number recall and memory for pictures. Both drugs produced dose-related increased in subject and observer ratings of drug effect.

At each dose level of pentobarbital, comparisons of the peak ratings of drug effect by subjects and staff observers were virtually identical. In contrast, while subjects and staff observers were in concordance as to the magnitude of the drug effect at the two lower doses of ethanol, subjects rated the peak drug 'effect for the highest dose of ethanol substantially lower than the peak rating of staff observers. In previous studies from this laboratory, similar under-rating of subjective drug effects by subjects at high doses with benzodiazepines, but not with other sedative-hypnotics.

For ethanol and pentobarbital, there were similar dose-related increases in subject ratings of sleepy and drunken and the subjects' retrospective ("Next Day") assesment of the previous day's drug effect and their estimate of the drug's street value. In general, subjects rated drug liking and retrospective "Next Day" liking substantially lower for ethanol than for the corresponding 600 and 750 mg doses of pentobarbital, even when the magnitude of the drug effect on other measures was roughly equivalent.

Pentobarbital produced significant dose-related increases in subject ratings on the mood dimensions of sluggish, confused, weary, drowsy and sleepy, while ethanol only produced significant increases in the mood dimension of confused. Observer's ratings showed corresponding dose-related increases in confusion for both ethahol and pentobarbital.

The results of this study document the well-recognized similarities in the behavioral and psychomotor performance effects profiles of ethanol, and other sedative-hypnotics, such as pentobarbital. However, ethanol's relatively modest same-day and next day drug liking scores, combined with the subjects' relative under-rating of ethanol's effects, suggests that, in addition to the many well known similarities of -ethanol to other sedative-hypnotics, there appear to be other important, and as yet poorly understood, differences.

Supported by NIDA Grant DA-03889.

AFFILIATIONS: Behavioral Pharmacology Research Unit
Department of Psychiatry and Behavioral Sci.
The Johns Hopkins University School of Med.
Baltimore, Maryland 21224

Subjective Effects of Methaqualone

Martin Ionescu-Pioggia, Michael Bird and Jonathan 0. Cole

Methaqualone (MQ) was widely abused until 1984 when it was placed in Schedule I. Jasinski et al. (1977) conducted the first laboratory evaluation of MQ comparing its subjective effects (SE) to those of pentobarbital in prison Ss. MQ produced SE similar to pentobarbital and was identified by Ss and barbiturate. The present study employed male and female, community resident, nonmedical substance users who were not drug-dependent to compare the SE of 2 doses of MQ (200, 400 mg) to placebo (P). Ss rated SE in groups in a naturalistic living room-like atmosphere mimicking the setting in which drugs are normally used. Two doses of MQ and P were administered to 4 groups of 6 Ss (n-24, 12M & 12F) in a double-blind randomized fashion. Ss' mean NMPI profile showed elevations on scales 4/Pd and 9?ma. SE were assessed predrug and hourly for 4 hours using ARCI List-l 16, and were analyzed by ANACOVA covaried for baseline. New ARCI factors and an Abuse Potential Scale (APS) were derived. Both doses of MQ were generally distinguishable from P ($p \leq .05$) on the following scales: Physical & Mental Sedation; Euphoria & Motor Stimulation; Physical Unpleasantness; AP; and, the standard Sedation-Euphoria scales. However, 200 mg was generally indistinguishable from 400 mg. Interestingly, both doses of MQ produced equivalent Euphoria with a dose related increase in Sedation. Results were unchanged when ARCI items were scored on a 6 point scale or true-false. Estimates of 'Street Value' discriminated both doses from P but did not discriminate between doses, with MQ 400, 200, and P valued at $2.43, $2.08. and $0.33. respectively. In summary, the breath of MQ's SE is striking. This study used a different method and S population to confirm earlier findings by Jasinski et al. (1977) that MQ is a sedative-euphoriant with high abuse potential.

AUTHORS

M. Ionescu-Pioggia, Ph.D. - Burroughs Wellcome Co.

M. Bird, Ph.D. and J.O. Coles, M.D. - McLean Hospital-Harvard Medical School

The Cocaine Expectancy Questionnaire (CEQ): Its Construction and Predictive Utility

Adam J. Jaffe and M. Marlyne Kilbey

The Cocaine Expectancy Questionnaire (CEQ) was developed to explore the domain of adult cocaine related expectancies. The questionnaire items were derived from extensive open ended interviews with 73 adult non-cocaine users, 12 experimental users and 20 abusers, as well as a review of the relevant literature. The items were then administered to a second, similar group, and item analysis was conducted to determine final item inclusion. A content analysis of the interviews and resulting questionnaire revealed that adults seem to hold well formed expectancies about the effects of cocaine, that include: paranoia, antisocial/aggressive behavior, grandiosity, desire for other drugs, and improvement or decrement in; sexual functioning, relaxation, and generalized physical functioning. Further, analyses were conducted on a matched sample of 20 non-cocaine users and 20 abusers. These groups were compared on two expectancy scales, cognitive and sexual functioning, considered from past research to be of greatest theoretical interest. Results indicate that cocaine abusers have significantly stronger expectancies of cognitive and sexual enhancement from cocaine use than do non-users ($P<.05$). Etiological implications, and the potential predictive utility of cocaine expectancies as measured by the CEQ are discussed.

AUTHORS:

Adam J. Jaffe, Ph.D.
Yale University
Alcohol Treatment Unit
New Haven, CT 06511

M. Marlyne Kilbey, Ph.D.
Wayne State University
Detroit, MI

Urinary Elimination Half-Life of Delta-1-Tetrahydrocannabinol-7-oic Acid in Heavy Marijuana Users After Smoking

Eva K. Johansson, Leo E. Hollister and Magnus M. Halldin

ABSTRACT

The urinary excretion of Δ^1-tetrahydrocannabinol-7-oic acid (Δ^1-THC-7-oic acid), the major urinary metabolite of Δ^1-THC, and the total amount of THC metabolites was studied in heavy marijuana users after smoking using high-performance liquid chromatography and the EMIT-d.a.u. cannabinoid assay. An average elimination half-life (±SD) of 3.0±2.3 days was obtained for Δ^1-THC-7-oic acid. The average ratio (±SD) of "EMIT readings"/ Δ^1-THC-7-oic acid concentrations was 1.23±1.03.

INTRODUCTION

Δ^1-Tetrahydrocannabinol (Δ^1-THC) is the major psychoactive compound in *Cannabis* . It is rapidly and extensively metabolized in man and only trace amounts are excreted in the urine (Agurell et al., 1986). Δ^1-THC-7-oic acid, the major urinary metabolite of Al-THC, has been determined by various analytical methods, such as high-performance liquid chromatography (HPLC), gas chromatography and gas chromatography/mass spectrometry (Cook, 1986). For forensic purposes, the total amount of THC metabolites is usually semiquantitated by enzyme multiplied immunoassays (EMIT) or radioimmunoassays (RIA) (Cook, 1986). In this study the urinary elimination half-life of Δ^1-THC-7-oic acid was determined in heavy marijuana users using HPLC. In addition, the Δ^1-THC-7-oic acid concentrations were compared to the total amount of THC-metabolites in the urine measured by the EMIT-d.a.u. cannabinoid assay.

METHODS

Thirteen males who were heavy *Cannabis* users volunteered to participate in this study. The subjects smoked four cigarettes containing with 15 mg Al-THC each during a two day period. In addition, the subjects were allowed to smoke marijuana in their regular manner. However, after the smoking period the subjects had to abstain from all *Cannabis* use for 28 days. Urine samples of 25 mL were collected in silanized glass tubes during the days of administration and on the first, second and third day after administration followed by every second day during the four week period of abstinence. The semiquantitative EMIT-d.a.u. cannabinoid assay was used to analyze the total amount of THC-metabolites in the urine. This system is designed to detect several metabolites of Δ^1-THC, being

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most sensitive to Δ^1-THC-7-oic acid. Concentrations of "Δ^1-THC-7-oic acid equivalents" were obtained using the calibration kits (cut-off level 20 ng/mL, medium level 75 ng/mL of Δ^1-THC-7-oic acid) supplied by Syva Scandinavia. The concentrations of Δ^1-THC-7-oic acid were determined by HPLC with UV and electrochemical detection after purification on Bond-Elut -THC columns (ElSohly et al., 1983). The detection limit of this method is 7 ng/mL urine. The elimination rate constant (λ) was calculated from the slope (λ/ln10), obtained by linear regression, of the data points in the urinary concentrations of Δ^1-THC-7-oic acid, divided by creatinine, versus time curves. The elimination half-life was obtained from $t_{½} = \ln 2/\lambda$.

RESULTS AND DISCUSSION

The urinary excretion of Δ^1-THC-7-oic acid in thirteen male heavy marijuana users was estimated after smoking a cumulative dose of Δ^1-THC during two consecutive days. The urinary concentrations of Δ^1-THC-7-oic acid were followed for up to 25 days by HPLC analysis. The acid concentrations were correlated to creatinine levels in order to adjust for variations in urine flow rate. Fifteen minutes after smoking the levels were 14-219 ng of Δ^1-THC-7-oic acid/mg creatinine. At 24 h and 3 days the levels had declined to 16-158 ng and 5-75 ng Δ^1-THC-7-oic acid/mg creatinine, respectively. The elimination half-lives ranged from 0.8-9.8 days with a mean (±SD) of 3.0±2.3 days. The elimination half-lives increased when the time period of analysis increased, which is in agreement with the multicompartment model. The EMIT-d.a.u. cannabinoid assay was used to analyze the total amount of THC metabolites excreted in the urine. The urinary levels of Δ^1-THC-7-oic acid were compared to the concentrations of "Δ^1-THC-7-oic acid equivalents" (within the linear range of 20-75 ng/mL) obtained by EMIT. An average ratio±S.D. of 1.23±1.03 for the EMIT readings/Δ^1-THC-7-oic acid concentrations was found. The poor correlation between the EMIT readings and Δ^1-THC-7-oic acid levels is a consequence of the EMIT assay being sensitive to several THC-metabolites excreted in urine.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Stig Agurell for valuable discussions. This project was supported by NIDA grant DA-03926.

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Eva K. Johansson[1], Leo E. Hollister[2] and Magnus M. Halldin[3]
[1]Department of Pharmacognosy, Box 579, BMC, S-751 23 Uppsala, Sweden.[2]Harris County Psychiatric Center, P.O. Box 20249, Houston TX 77225-0249, USA.[3]Department of Pharmacology, Karolinska Institute, S-10401 Stockholm, Sweden.

Use Predicts Treatment Outcome, Not Opiate Dependence or Withdrawal

Therese A. Kosten, Mark S. Bianchi and Thomas R. Kosten

The dependence syndrome concept, proposed by Edwards and Gross (1976) for alcohol, was extended to other drugs and is the basis of DSM-III R criteria for drug dependence. We showed that these behavioral and physiological aspects of dependence were related to biological addiction in opiate addicts: greater opiate dependence associated with more severe naloxone precipitated opiate withdrawal (Kosten, et al, in press). Neither opiate dependence nor withdrawal severity was related to frequency or length of opiate use. Moreover, we found that cocaine dependence (DSM-III R) predicted less severe opiate withdrawal. To test further the validity of the dependence syndrome concept in opiate abuse, we investigated whether the degree of opiate dependence would predict treatment outcome in a methadone maintenance program. We also examined the predictive utility of the other drug use and demographic variables.

METHOD

Fifty-two of 48 opiate addicts were re-interviewed 1 year after the start of methadone maintenance treatment (92% follow-up rate). The subjects tended to be male (69%), white (67%), and were 32.1 ± 1.0 years old. At intake, we assessed opiate and cocaine dependence using the Structured Clinical Instrument for Diagnoses (SCID) and gathered additional information on drug use history including length and frequency of use and number of previous treatment attempts. Frequency of opiate use was obtained by a self-report using a 6 point scale where 6 was the most frequent use. Opiate withdrawal severity was rated using the Naloxone Challenge Test. These variables, along with demographic variables, were used to predict success in treatment, as defined by treatment length (weeks) and percent illicit urines.

RESULTS

Demographic predictors. Gender did not predict treatment length nor illicit urines. More illicit opiate urines were seen in older addicts but, age was not related to any other success variable. Finally, black addicts had higher percent of illicit cocaine urines (53%) than white addicts (15%; $p<0.05$).

Drug use predictors. Overall, naloxone-precipitated opiate withdrawal was not associated with treatment success nor was opiate dependence. Cocaine dependence predicted a greater number of illicit cocaine, but not opiate, urines ($r=0.37$, $p<0.01$) and tended to predict shorter treatment length ($r=-0.25$, $p<0.1$). Neither years of opiate use nor number of past treatments predicted treatment success. The best predictor of treatment success was self-report of opiate use frequency, where greater opiate use was associated with shorter treatment length ($r= -0.46$, $p<0.01$).

Predictive model. Stepwise regression. of all variables emphasized the predictive value of opiate use on treatment length (partial R^2 =0.21). Since race also added to the model (partial R^2 =0.05), we analyzed the association between intake variables and treatment success separately by race. Treatment length was predicted extremely well by drug use in blacks ($r=-0.66$) and by both drug use ($r=-0.43$) and opiate dependence ($r=-0.31$) in whites. The stepwise regression model for whites also included opiate withdrawal severity as a modest predictor of outcome (partial R^2 =0.06).

DISCUSSION

We found that opiate dependence and opiate withdrawal severity predict treatment success modestly in white opiate addicts only. Yet, self-reports of drug use frequency are very good predictors of treatment outcome, especially in black opiate addicts. Whether the self-report reflects the true frequency of use or is a measure of perceived drug severity is not known, but it provides an easy-to-use and very good predictor of treatment success.

The other factors that predict failure in methadone maintenance treatment are: 1) greater cocaine dependence: 2) being older; and 3) being nonwhite. However, none of these varibles predicts treatment outcome as well as the self-report of opiate use.

From the Dept. of Psychiatry, Yale University Medical School. Supported by NIDA grant P50DA04060.

Buprenorphine Treatment of Cocaine Abuse

Thomas R. Kosten, Charles J. Morgan and Herbert D. Kleber

Intravenous cocaine abuse among opioid addicts has become a major public health problem that may be reduced by buprenorphine, an opioid mixed agonist antagonist. This open study compared cocaine abuse in buprenorphine (BUP) to methadone (METH) maintained patients using two designs: 1. cross-over, 12 METH patients at a mean METH dose of 47 (± 8 mg PO) were switched over to BUP dose of 3.8 (±0.6) mg SL for 1 month. On METH they had cocaine in 20% of their urines over the previous 6 months, but on BUP their cocaine positive urines dropped tenfold to 2% (t=2.8, P 0.02). 2. In the case control, 41 BUP (3.2 ± 1.6 mg SL) were compared to 61 METH (43 ± 8 mg PO) and 36 naltrexone (NLX) patients for the first month in treatment. The groups were comparable in age, sex, race and number of urines per patient (mean = 8.4). Urines were positive for cocaine in 24% of the METH and in 3% of the BUP group (t=5.8, df=71, P 0.0001), but overall illicit urines did not differ (33% METH vs. 37% BUP). The NLX patients showed no difference from the BUP patients, but significant difference from the METH patients in cocaine abuse (5% Positive urines) (F=17.95; df=2,137; P 0.0001). Thus, BUP and opioid antagonists in general may be a more effective treatment than METH for cocaine abusing opioid addict.

AFFILIATION:

Department of Psychiatry
Yale University School of Medicine

Marijuana and Alcohol Effects on Mood States in Young Women

B. W. Lex, M. L. Griffin, N. K. Mello and J. H. Mendelson

Potential predictors of 8 profile of Mood States (POMS) factor scores were examined for 30 young women (mean age = 26.4 years) who smoked marijuana and drank alcohol in the community. Prospective data were obtained from diary questionnaires submitted daily during 3 consecutive menstrual cycles. Behavioral and social variables (heavy [≥ 1.5 cigarettes per day] versus light [< 1.5 cigarettes per day] marijuana smoking, use of both marijuana and alcohol on the same day, occurrence of unusual events, and participation in sexual activity) were strong predictors for mood factors than were temporal (weekdays versus weekends) or biological (menstrual cycle phase) variables. Heavy marijuana users consistently reported higher negative (confusion, anger, fatigue, and tension) and lower positive (friendliness, vigor, and elation) mood scores. This study was supported in part by Grants DA 02905, DA 04059, DA 00101, and DA 00064 from NIDA and Grants AA 0652 and AA 06794 from NIAAA.

Alcohol and Drug Abuse Research Center, Harvard Medical School, McLean Hospital, Belmont, MA 02178

Microanalysis of Ethanol-Induced Disruption of Body Sway and Psychomotor Performance in Women

B. W. Lex and S. E. Lukas

Repeated measures of body sway, psychomotor performance, and blood alcohol level (BAL) were obtained from 20 women after they received either ethanol (0.56 g/kg) or isocaloric placebo. Simple motor tasks (finger tapping and hand steadiness) were unaffected by this dose. Performance on a computer-automated version of the Digit Symbol Substitution Test was disrupted only during the ascending portion of the BAL curve. Body sway (static ataxia) was affected during both peak and descending BALs. A platform device connected to a microcomputer measured static ataxia, and a customized software program registered the frequency and extent of movements during each 60 second test interval. Body sway data following placebo or ethanol were plotted in three-dimensional space. Analysis of dynamic changes showed more pronounced disruption in the sagittal (anterior-posterior) plane than in the lateral plane. Subjects swayed more to the posterior and to the right. Results suggest that the apparatus and data analysis techniques provide highly sensitive measures of body sway following a moderate dose of ethanol. This study was supported in part by Grant AA 06794 from NIAAA.

Alcohol and Drug Abuse Research Center, Harvard Medical School, McLean Hospital, Belmont, MA 02178

Behavioral Effects of Alprazolam in Chronic Therapeutic Users

I. Lucki, L. A. Kerr, R. B. Frldman, E. Schweizer and K. Rickels

This study presents initial preliminary data of a comparison of the behavioral effects of the benzodiazepine alprazolam in chronic medical users and normal volunteers. Doses of 1 or 2 mg of alprazolam or placebo vere administered double-blind to a group of 6 chronic users and 6 normal volunteers. The chronic users (5 females, 1 male; 39.8 ± 3.2 years) vere taking alprazolam at therapeutically-appropriate doses on a regular daily basis for at least 1 year for the treatment of panic disorder (Dose = 2.1 ± 0.6 mg; Duration = 3.2 ± 0.6 years). The normal volunteers (N=6) were matched for sex (5 females, 1 male), age (42.7 ± 2.8 years), and educational level vith the group of chronic users. No volunteers or patients had a history of treatment for a substance abuse disorder. All subjects were tested on a battery of tests of psychomotor performance (tapping, DSST, symbol copying, CFF threshold), verbal recall ability, subjective effects, and drug-liking scales.

Preliminary results from the psychomotor tests suggest that alprazolam produced somevhat less impairment of psychomotor performance in chronic users than in drug-naive normal volunteers. Amnesic effects of alprazolam vere measured in both chronic users and in normal volunteers. Normal volunteers reported increased feelings of mental and physical sedation with increasing doses of alprazolam. Normally high level of tranquilieation vere not changed by alprazolam in normal volunteers. Chronic users did not report increases in mental or physical sedation vith alprazolam. although baseline values for this group vere somevhat higher. Alpraeolam appeared to increase the report of tranquilization in chronic users. Normal volunteers accurately discriminated alprazolam from placebo. Chronic users incorrectly reported receiving alprazolam vhen they vere actually given placebo. Alprazolam appeared to produce a moderate degree of drug-liking in both normal volunteers and chronic users. Supported by DA 05186.

AFFILIATION

Departments of Psychiatry and Pharmacology. University of Pennsylvania, 207 Piersol Building, Philadelphia, PA 19104-4283.

Naltrexone in Federal Probationers

David S. Metzger, James Cornish, George E. Woody, A. Thomas McLellan, Patrick Druley and Charles P. O'Brien

Introduction

The opiate antagonist naltrexone hydrochloride has been proven to be a safe and effective medication in blocking the actions of opiates and preventing dependence. Naltrexone has few side effects and individuals taking this medication do not become dependent upon it. Its clinical utility however, has been limited by the general lack of *acceptability* of the medication among many patient populations. This poster presents some preliminary findings from a unique treatment program for individuals at risk of incarceration should they relapse to opiate addiction.

The Naltrexone Treatment Program is designed to determine the feasibility and effectiveness of naltrexone when used with individuals at risk of incarceration should relapse occur. It is believed that this group represents a more highly motivated patient population than may be available in other settings.

The Program

The program is a project is located in the Federal Probation office in Philadelphia. Although the research is administratively autonomous from the probation office, a close working relationship with the probation staff is maintained. We offer probationers a convenient opportunity for treatment which would otherwise be unavailable.

The project has been operational for the past eighteen months. The Naltrexone Treatment Program is staffed with a full-time substance abuse research technician. A physician is on call at all times and provides weekly on site coverage. All subjects are provided with medical screening and monitoring as well as weekly drug counseling.

Design and Methods

The program is being evaluated using an open, randomized control group design. The use of a placebo control group was determined to be inappropriate given the ability of patients to easily identify their medication status through opiate use following random assignment. Participation in the research is completely voluntary. Individuals with histories of opiate dependence or who are evidencing early signs of relapse are self referred or referred by their probation officer. The project staff provide a full description of procedures and potential risks and benefits of participation.

Following orientation and informed consent, volunteers are randomly assigned to either the medication group or the control group. Those assigned to the medication group are first challenged with naloxone to insure that they are free of opiates. Following a successful naloxone test, subjects are administered their first dosage of naltrexone. Subsequently, naltrexone patients receive medication two times each week in order to provide adequate blockade coverage.

Those individuals assigned to -the control group are also scheduled to visit the program two times each week for counseling and monitoring. All subjects are asked to contact their probation officers each visit. Drug use (self report and urinalysis) and drug craving are monitored on a weekly basis as are physical and psychological symptoms, treatment involvements, and social functioning.

Preliminary Results

Twenty four subjects have completed the six month study phase of the project. Preliminary results indicate that the six month retention rate for the probationers randomly assigned to the naltrexone group is fifty percent. There have been few negative side effects noted by patients taking the medication. The greatest challenge facing the project is the current low rate of identifiable opiate abuse and the epidemic use of cocaine. This situation not only limits the number of subjects available for study but also highlights the project's need to address issues of nonopiate substance abuse. Of perhaps equal importance is the fact that our findings demonstrate that the delivery of services within a probation environment is a viable model for drug abuse treatment delivery. Many probationers experience significant difficulty with drug use and the delivery of services within the probation environment has met with acceptance from both staff and patients.

Center for Studies on Addiction
University of Pennsylvania/Philadelphia Veterans Administration Medical Center

The Carrier Addition Severity Index for Adolescents (CASI-A)

K. Meyers, J. Jaeger, D. Metzger and P. Sargiotto

The Carrier Addiction Severity Index for Adolescents (CASI-A), modeled after the Addiction Severity Index (ASI), is a 45-60 minute semi-structured interview which assesses eight areas critical to comprehensive adolescent assessment and treatment outcome evaluation: medical, education, substance use, use of free time, peer relationships, family relationships, legal status, psychiatric status. During the initial stage of development, the CASI-A was piloted on three populations: adolescents receiving inpatient substance abuse treatment at a psychiatric facility, adolescents receiving education at a private school for emotionally disturbed students, and adolescents referred to the Juvenile Justice System of Pennsylvania. Substantial revisions in ASI content and the scoring system were necessary to capture the unique characteristics of the adolescent population.

Preliminary data on the most recent version of the CASI-A (N = 25 adolescents receiving substance abuse treatment at a psychiatric facility) indicates a lack of agreement between adolescent objective data and their subjective ratings in select areas. For example, 68% of the adolescents who disclosed positive symptomatology (e.g., used a drug regularly, frequently went to class under the influence) reported "no problems" with substance abuse. 50% of the adolescents who disclosed positive symptomatology (e.g., failing grades, truancy, contemplating dropping out) reported "no problems" with education. This suggests that adolescents do not always have insight into the severity of their problems and have difficulty making judgements regarding the importance of treatment. Their subjective ratings should, therefore, be viewed with caution. Objective data in concrete areas has proven most useful in assessing of this population. Analysis of problem onset has also proven beneficial to

overall assessment. For example, education and psychiatric problems began earlier than substance abuse problems (X= 5.7 grade onset education problems vs. X = 7.5 grade onset substance abuse problems, t = 2.69, p = .014; X = 5.0 grade onset psychiatric problems vs. X = 7.5 grade onset substance abuse problems, t = 2.40, p = .035), while there was no significant difference in problem onset of other areas in comparison to substance abuse.

Like the ASI, the CASI-A has both clinical and research applications. The type of infomation obtained is purposely focused to assist clinicians in identifying treatment needs, determining individual treatment plans or intervention referral, and monitoring change over time. In fact, clinicians have found the instrument beneficial during intake interviews and adolescents have commented positively about its content, format, and usefulness to treatment. Use of the instrument should allow researchers to develop treatment-matching criteria, evaluate the success of treatment-matching procedures, and conduct treatment outcome studies. Ultimately, use of the instrument should assist clinicians in matching adolescents to proper treatment, and should facilitate greater comparability of research results.

CARRIER FOUNDATION, NEW JERSEY
UNIVERSITY OF PENNSYLVANIA, PENNSYLVANIA

Metabolic Effects of Nicotine in Smokers and Non-smokers

Kenneth A. Perkins, Leonard H. Espstein, Richard L. Stiller, Joan E. Sexton and Rolf G. Jacob

INTRODUCTION

Cigarette smoking is inversely related to body weight, as smokers weigh less than nonsmokers and gain weight after stopping smoking. Little evidence indicates that smokers consume fewer calories or are more physically active than nonsnokers, suggesting that the metabolic effects of smoking (more specifically, nicotine) may be important in explaining the differences in body weight as a function of smoking status. We recently found that nicotine presented in spray form, isolated from tobacco smoke, produces a significant acute increase in metabolic rate in cigarette smokers. The present study compared the acute metabolic effects of nicotine between smokers and nonsnokers to help determine whether chronic tolerance may occur to these effects. Chronic tolerance would indicate that the metabolic effect is more prominent when smokers initially adopt the smoking habit but plays a lesser role in determining body weight in regular smokers. A nasal spray dosing procedure, which produces rapid dose-dependent boosts in plasma nicotine similar in magnitude to smoking, was employed to standardize dosing across subjects, particularly between smokers and nonsmokers.

METHODS

Subjects were male smokers and nonsmokers (n=l0 each) matched on age (23.2 vs. 21.7 yrs, respectively), body weight (74.8 vs. 75.5 kg), and physical fitness (38.4 vs. 41.2 mlO_2/kg/min). Smokers smoked an average of 19.8 cigs/day for 5.1 years. Metabolic rate was assessed continuously via computerized indirect calorimetry. Nicotine and placebo ware presented double-blind using a measured-dose nasal spray system developed in our lab. The nicotine dose was 15 ug/kg, approx. 1.1 mg (typical intake of smokers from an average cigarette) for the average-weight subject in this study.

Subjects participated in 2 sessions on separate mornings, each after overnight abstinence from smoking, caffeine, and food. Subjects remained at rest in a comfortable reclining armchair throughout each session. Following 30-min baseline determination

of resting metabolic rate (RMR), subjects were presented with nicotine or placebo every 20 mins for 2 hrs, with metabolic rate assessed between presentations. Order of nicotine vs. placebo was counter-balanced between the 2 sessions, arid the experimenter was blind to subjects' smoking status.

RESULTS

There were no effects of group or session on baseline RMR, prior to nicotine or placebo (mean±SEM for all subjects = 1.017±.019 kcal/kg/hr . Energy expenditure of smokers and nonsmokers was significantly increased by nicotine intake ($p<.01$), but there was no difference between smokers and nonsmkers. For smokers, the man increase above baseline due to nicotine was .069±.019 kcal/kg/hr, or 6.8% above RMR, while nonsmokers showed an increase of .063±.014 kcal/kg/hr, or 6.4% above RMR.

The energy expenditure of nonsmokers increased slightly following placebo (.032 kcal/kg/hr, 3.2% above RMR). We have observed placebo responses of the same magnitude in smokers in previous studies and have found that they appear to be due to effects of inhaling, similar to behavioral aspects of making. However, we did not observe a similar increase following placebo in the smokers of this study (.010 kcal/kg/hr, 1.0% above RMR), but therewasno significant interaction between smoking status and nicotine/placebo on metabolic response.

DISCUSSION

To our knowledge, this is the first study directly comparing metabolic responses to a measured dose of nicotine between smokers and nonsmokers. Our finding of no difference in response to nicotine between smokers and nonsmokers suggests that chronic tolerance does not occur to the metabolic effectsof nicotine. Thus, there does not appear to be physiological adaptation to the metabolic effects of nicotine with chronic nicotine intake (i.e. smoking). In addition to lack of difference in response to nicotine, there was no difference in baseline between smokers and nonsmokers, similar to our previous studies. These results further support the notion that there is no long-term effect of smoking on metabolic processes in the absence of recent nicotine intake. Further research should determine whether lack of differences in RMR and response to nicotine also occurs with older smokers with longer smoking histories and with females, who generally experience greater differences in eight due to smoking.

Supported by R01-04174 from the National Institute on Drug Abuse.

AFFILIATION: Western Psychiatric Institute &Clinic
University of Pittsburgh School of Medicine

Differential Anxiety Symptoms in Cocaine vs. Alcoholic Patients

H. Pettinati, B. Evans, C. Joseph, J. Jensen and K. Meyers

Patients presenting for substance abuse treatment typically exhibit a variety of symptoms of psychopathology including anxiety and depression. However, following four weeks of treatment where abstinence has been maintained throughout, the majority of these symptoms will subside except when a premorbid psychiatric disorder exists. As part of a large assessment battery, the Clinical Anxiety Scale (CAS), the Hopkins Symptom Checklist-9Q (SCL-90-R) and Hamilton Depression Rating Scale (HAM-D) were administered to 50 patients within a week of presenting for inpatient substance abuse treatment. Of the 50 patients, 25 met DSM-III-R criteria for alcohol dependence, with no other concomitant drug abuse or dependence, and 25 met DSM-III-R criteria for cocaine dependence and alcohol dependence or abuse. Reassessment of psychopathology occurred 4 weeks later, prior to discharge, at which time patients were also assessed for current and lifetime Axis I psychiatric disorders and Axis II personality disorders using the Structured Clinical Interview for DSM-III-R (SCID).

Preliminary analyses show differences in the extent of certain types of psychopathology between patients dependent only on alcohol versus those dependent on cocaine and alcohol. For example, prior to discharge (at Week 4), more patients in the cocaine and alcohol group compared to the alcohol only dependent group met criteria for an Axis I (20.8% vs. 12.5%, respectively) or Axis II (60% vs. 28%, respectively) disorder. This difference was statistically significant for Axis II psychopathology (Chi Square= 7.62, df = 2, $p<.05$).

At the time of discharge, while both groups showed a decrease in overall psychopathology on the SCL-90-R. the

cocaine and alcohol dependent group showed a significantly smaller decrease ($F = 4.3$, $p < .05$) in psychopathology and scored significantly higher than the alcohol only dependent group. The mean total SCL-90-R scores were significantly higher for the patients in the alcohol and cocaine dependent group [X SCL-90-R (Week 4) = 60.0 vs. 34.3, respectively], although the mean total SCL-90-R scores at Week 1 were not different between the two groups (X SCL-90-R (Week 1) = 87.3 vs. 83.3, respectively).

While mean total CAS scores did not differ between patient groups at Weeks 1 or 4, an item analysis on the CAS revealed differences in types of symptoms between the two patient groups, both at Week 1 and 4. Those dependent only on alcohol were rated higher (increased anxiety) on psychic tension ($p=.03$) than those patients dependent on both cocaine and alcohol. However, the cocaine and alcohol dependent group scored significantly higher (increased anxiety) on motor restlessness ($p=.03$). At discharge, although a diminution in symptoms of anxiety on the CAS had occurred in both groups, motor restlessness continued to significantly distinguish the two groups with significantly more restlessness being reported by those dependent on both cocaine and alcohol ($p=.01$).

Preliminary data are also presented that illustrate the interface among severity of psychopathology, severity of craving, and nature of the personality disorder with regard to the probability that the patient will relapse.

AFFILIATION:

Carrier Foundation
New Jersey

Precarious Dilemmas: Mobilizing Blacks Against AIDS

Ernest Quimby

INTRODUCTION

AIDS has disproportionately affected blacks, nationally and in N.Y.C. Black organizations have been relatively slow in responding to the HIV epidemic, however, because they encounter several precarious dilemmas in their philosophy, constituency, outreach practices, and limited resources.

RESEARCH QUESTIONS

How are black organizations responding to the disproportionate effects of the HIV epidemic on blacks?

What are the implications of black organizational response for intervention and prevention efforts?

METHODS

Qualitative data were gathered by ethnographic methods in 1987-88. Author attended meetings of predominantly black groups, including conferences, street rallies, political forums and AIDS awareness events in churches, universities, hospitals/clinics, parks, drug treatment centers, and youth programs, mainly in New York, Atlanta, Washington, D.C., New Jersey, Philadelphia, and Boston. Observations were conducted of their mobilization and outreach activities. Formal and informal interviews occurred. Additional material was compiled from media and scientific reports, field notes, transcripts, training and educational sessions, and related events.

CENTRAL FINDINGS/DISCUSSION

The structure of African American neighborhoods relies on governmental aid and external assistance which may foster conservatism and conflicting attitudes towards AIDS.

AIDS & HIV have become metaphors representing the problematic existence of the African American organizations and communities: uncomfortable racial and ethnic relations, economic insufficiency, subordination and dependency, threats to acceptable struggles for social mobility and community development, and stigmatization of being associated with AIDS.

Organizational effort against AIDS by some black churches and health care organizations has been limited to meetings, rallies, & conferences which have self-selected audiences. limited attendance, and little sustained follow-up. African American organizations and their constituencies tend to shift responsibility and avoid commitments to confront HIV issues by focusing on conspiracies by whites, and moral condemnation of addiction and sexual promiscuity. Official or private efforts to contain HIV by needle exchanges or methadone maintenance are sometimes viewed as oppression and tacit approval of morally condemned behavior.

Organizational efforts by professional elites are aimed at their constituencies rather than neighborhoods. Because of the immediate and direct danger to their constituents, drug treatment programs have mobilized faster than churches and health care organizations. Other than treatment programs, mosts organizations are unclear about their role in preventing AIDS.

The dilemmas of African American organizations are reflected in several ways: infrastructural weaknesses, minimal resources, inability to compete for massive AIDS funding, and relatively limited fundraising capabilities. Also, most black groups were formed for purposes other than HIV intervention and education. AIDS is frequently either considered offensive and/or of secondary or minor importance to such organizations.

CONCLUSIONS/IMPLICATIONS

Black institutions and subcultures have not as readily responded to AIDS as white gays, Black organizations face major dilemmas and constrains: limited availability and control of economic and political resources, relative decline of Black liberation movement, ideological confusion, cleavages by class, religion and cultural origin and epidemiologically different risks of exposure to HIV.

Public health for and in black communities is a socio-economic and political issue. Public health policy, however, is shaped and influenced by definitions, agendas, institutions, cultures and political activity primarily outside the black community.

Mobilization requires that a community be pre-disposed or ‘readied’ for mobilization. Effective HIV outreach needs to consider not only information and attitudinal change, but community development which allows its members to identify, confront, and adapt to HIV and other new problems.

Activation, recruitment, and innovative service delivery from the ‘bottom up’ may be more effective than traditional ‘top down’ medical approaches in stemming the disease’s spread.

AUTHOR:

Ernest Quimby, Ph.D.
Howard University
Washington, D. C. 20079

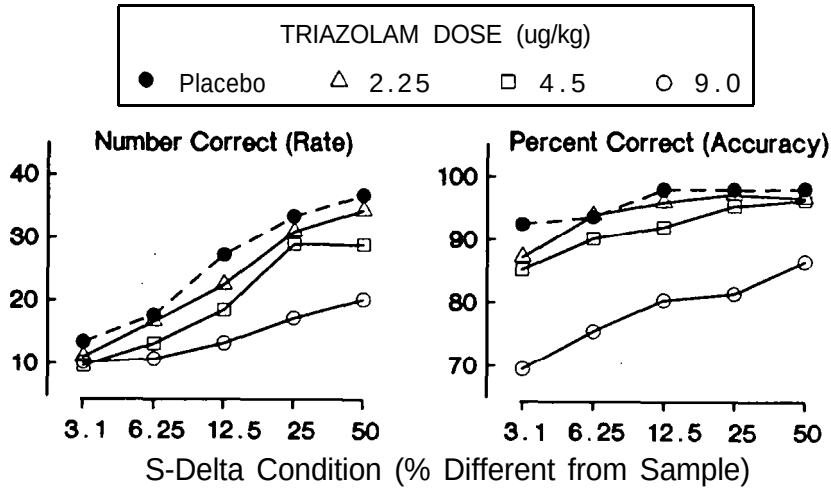
Effects of Triazolam (TZ) on Matching-to-Sample (MTS) Performance in Humans

J. D. Roache, D. R. Cherek, K. A. Cowan, R. Spiga, R. H. Bennett and J. Grabowski

It is widely recognized that benzodiazepines impair cognitive and psychomotor task performanc in humans. However, the nature of these behavioral disruptions has not been precisely identified. The present study is a preliminary investigation of a computerized MIS task designed to permit a beavioral analysis of drug effects on a number of variables thought to be important influences on human behavior and performance.

The MTS task utilized checkerboard-like sample (SD) patterns displayed an a video screen and created by randomly darkening 32 (50%) of the squares of an 8 x 8 square matrix. Concurrently presented below the SD, two comparison stimuli were a copy of the SD and an S-Delta created by randomly varying the position of 1,2,4,8 or 16 of the SD squares. Thus, the five S-Delta conditions were 3.125, 6.25, 12.5, 25 or 50 percent different from the SD. Each task performance session was a multiple component schedule consisting of fifteen 60 sec segments separated by a 15 sec time out. A 60 sec trial of each of the five S-Delta conditions occured within each of 3 random blocks. Subjects responded on left or right manipulanda buttons to match the SD pattern. Correct responses resulted in a brief tone presentation. At the end of the day, subjects were paid $0.01 for each point derived as the total number of correct responses minus errors (approximately $20).

The present study examined the effects of placebo and three TZ doses (2.25, 4.5 and 9.0 ug/kq) administered orally under double-blind conditions at 0930 hr. Seven normal healthy male subjects participated for 8 hrs daily, three days per week. Subjects provided drub-free urine samples and alcohol-free breath samples daily and were required not to eat solid food or drink caffeinated beverages in the morning of each study day. MTS task performance occurred before drug administration and repeatedly at 0.5, 1.5, 3.0, 4.5 and 6.0 hrs following drug administration. Subjects received no drug on the first day and placebo for the next 4 days to allow for baseline performance acquisition. Subsequently, TZ doses were administered in an ascending series; usually on consecutive sessions.



Stable levels of performance were generally observed within the 5 day baseline period. Response rates and accuracy were an orderly function of the SD/S-Delta difference (i.e., discriminability). With placebo treatment, mean response rates per 60 sec trial ranged from approximately 15 for S-Delta=3.125% to 40 for S-Delta=50% and mean accuracy ranged from approximately 92% to 99%, respectively. Responses in one component of the muiltiple component schedule were not signficantly affected by the S-Delta condition of the preceding component. Overall, these data indicate that subjects were under reasonably good SD stimulus control. However, compared to the easier SD/S-Delta discriminations, the more difficult discriminations engendered slower response rates with less complete stimulus control.

The above figure shows that at 1.5 hr post-drug, TZ produced dose-related decreases in response rate (No. Correct). This occurred as an increasing function of the S-Delta difference from the sample (SD) resulting in a significant ($p<.001$) interaction of dose and S-Delta condition. Only the highest TZ dose ($p<.001$) disrupted accurate MS performance and this occurred across all S-Dalta conditions. TZ tended to produce greater disruption under the weaker stimulus control conditions of the more difficult discriminations (i.e., S-Delta = 3.125%). These results extend, into the analysis of performance impairment, previous work suggesting that drug effects depend upon baseline response rates and the degree of stimulus control of behavior.

Supppрted by NIDA Grant DA-05716

AFFILIATION: Substance Abuse Research Center, UT-MSI, Department of Psychiatry & Behavioral Sciences, University of Texas Health Science Center at Houston, Houston, TX 77030

Human Multioperant Responding: Effects of Triazolam

Ralph Spiga, Don R. Cherek, Richard A. Meisch and John D. Roache

Although aversive stimuli occasion escape, avoidance, or aggressive responses in humans, studies of human aggressive behavior have limited the subject to aggressive responding. In this study we examined the effects of triazolam on human aggressive and escape responses occasioned by point subtractions attributed to another person.

Four male volunteers were recruited and screened. Those volunteers who had current medical or psychiatric problems including a history of alcoholism or substance abuse were excluded.

Subjects participated in six 25 minute sessions each experimental day. The sessions occurred immediately before dose administration, and at 1/2 hour, 1 hour, 2 hours, 4 hours, and 6 hours after administration of placebo, O.l25mg/70kg, 0.25mg/70kg, or 0.5mg/70kg of triazolam. The three doses were first administered in an ascending dose sequence and then in a randomly ordered dose sequence. Successive triazolam doses were separated by 72 hours.

Subjects were seated at a console consisting of three levers marked A, B, and C, and a numeric counter. Pulls on lever A, the point-maintained response, were maintained by an FR1OO schedule of point presentation. At random intervals points were subtracted from the counter ostensibly by a second subject. Ten (10) lever B pulls, the aggressive response, ostensibly subtracted points from this person's earnings. By pulling lever C, the escape response, subjects protected their counter from point loss for a period of time. Lever B, aggressive, or lever C, escape, responses which followed a point loss postponed point loss for 125s.

Subjects were told that the research project concerned the effects of triazolam on physiological and motor responses. They were told that pulling lever A 100 times would accumulate points exchangeable for money at the end of the day. Subjects were

informed that periodically during-the session another "person" with whom they were paired might subtract points from their counter. They in turn had the option of subtracting points from this person by pulling lever B 10 times or protecting their counter for a period of time by pulling lever C 10 times.

For each dose day and dependent measure the rates from the session which deviated most from placebo values were selected as data points. In one subject triazolam produced dose-dependent decreases in the rate of point-maintained responding. In all other subjects the lower doses of triazolam did not change or slightly increased the rate of point-maintained responding while the 0.5mg/70kg dose decreased the rate of-point-maintained responding.

Three of the four subjects responded on the aggressive response option. Dose-dependent decreases in the rate of aggressive responding were observed in one subject. Only at the highest triazolam dose was the rate of aggressive responding decreased in the other three subjects. The 0.25mg/70kg dose increased the rate of aggressive responding of the one subject who responded on both the aggressive and escape response option.

Two of the four subjects responded on the escape option. Dose-dependent decreases in the rate of escape responding were observed in one subject. Only the highest triazolam dose (0.5mg/70kg) decreased the rate of escape responding in the second subject.

Acute administration triazolam decreased point-maintained and aggressive responding. This effect is similar to effects observed following acute diazepam administration. Triazolam also decreased escape responding. Aggressive and escape responding decreased more than point-maintained responding, even at doses that had little or no effect on responding maintained by point presentation.

Our results indicate that benzodiazepines not only decrease aggressive responding occassioned by provocation and maintained by periods free of provocation, but also suppress escape responding initiated and maintained under the same conditions. Thus, benzodiazepines may disrupt the relationship between aversive stimuli (point loss) and aggressive and escape responding.

Acknowledgement: This research was supported by NIDA grant 03166 and NIDA Post-doctoral Fellowship 05369.

Affiliation: Substance Abuse Research Center
Department of Psychiatry & Behavioral Science
The University of Texas Mental Sciences Institute
The University of Texas Medical School
Houston, Texas 77030

The Acute Effects of Codeine on Human Aggressive and Non-Aggressive Behavior

Ralph Spiga, Don R. Cherek, John D. Roache and Kathy Cowan

This study examined the effect of codeine on aggressive behavior in a controlled laboratory setting.

Ten male volunteers were recruited by advertisement, screened, and excluded if they had current medical illness or history of psychiatric disorders including alcoholism or substance abuse.

Sessions were conducted Monday through Friday. Thirty minutes prior to sessions subjects were administered placebo or one of three codeine doses of 25mg/70kg, 50mg/70kg, and 75mg/70kg. Doses were administered first in an ascending sequence and then twice in a randomly ordered sequence.

Two responses were concurrently available as non-reversible options. Responses on button A earned points exchangeable for money on a FR 100 schedule. Responses on button B subtracted points exchangeable for money from another subject on a FR 10 schedule. Superimposed on the schedule of monetary reinforcement was a schedule of point loss. Points were subtracted at random times throughout the session unless the subject responded aggressively after a point loss. Aggressive responses following at least one point loss initiated an interval either of 125s or of 500s during which no points were subtracted.

After administration of the last dose subjects completed the Buss-Durkee Hostility Inventory. This inventory assessed history of assaultive behavior, irritability, indirect hostility, negativism, resentment, and verbal hostility.

Subjects were told that the research project concerned the effects of drugs on physiological and motor responses. They were told that Button A presses would accumulate points exchangeable for money and that periodically during the session another "person" might subtract points from their counter. They had the option of subtracting points from this person by pressing button B.

The frequency of monetarily reinforced, non-aggressive responding was unaffected by acute administration of codeine. The aggressive responding per provocation, expressed as a percent change from the placebo session preceding the acute administration of a codeine dose, exhibited the greatest increase for 6 of 10 subjects at the 50mg/70kg dose of codeine ($F\ 2, 16 = 4.50$; $p < .05$). Effects attributable to group or dose repetition were not significant.

Subjects were divided into two groups using their scores on the Buss-Durkee Hostility Inventory as the basis for assignment to a "High hostility" or a "Low hostility" group. We found an interaction of high or low hostility with dose ($F\ 2, 16 = 3.88$; $p.<.05$). The 25mg/70kg dose decreased, relative to placebo, the frequency of aggressive responding per provocation of high hostility subjects while increasing the frequency of aggressive responding per provocation of low hostility subjects. The frequency of aggressive responding per provocation was increased relative to placebo for both high and low hostility subjects at the 50mg/70kg dose. Acute administration of 75mg/70kg of codeine did not affect the frequency of aggressive responding per provocation of either the high or low hostility group.

The major findings of this study were that acute administration of a 50mg/70kg dose of codeine increased the frequency of aggressive responding per provocation; that acute administration of codeine did not effect monetarily reinforced responding; and that acute administration of a 25mg/70kg of codeine decreased aggressive responding per provocation of those subjects who reported that they tended to engage in verbally hostile or physically assaultive behaviors in the natural environment while increasing aggressive responses per provocation in those subjects who reported that they were not prone to verbal hostility or physical assaultiveness.

We conclude that acute administration of codeine increases aggressive responding following provocation and that this may be influenced by the subject's previous history of aggressive responding.

Acknowledgements: This research was supported by NIDA grant 03166 and Ralph Spiga was supported by NIDA Post-doctoral Fellowship 05369.

Affiliation: Substance Abuse Research Center
Department of Psychiatry & Behavioral Science
The University of Texas Mental Sciences Institute
The University of Texas Medical School
Houston, Texas 77030

Anterior Pituitary, Gonadal and Adrenal Hormones in Women with Alcohol and Polydrug Abuse

Siew K. Teoh, Barbara W. Lex, Joshua Cochin Jack H. Mendelson and Nancy K. Mello

Chronic alcoholism and drug abuse are often associated with derangements of reproductive function in women such as amenorrhea, anovulation, luteal phase dysfunction and early menopause. Endocrine profiles of the first 18 consecutive women (age 17-58) admitted to a Massachusetts hospital for treatment of alcohol/polydrug abuse under civil commitment (Section 35A of Chapter 123 of the general laws of Massachusetts) were studied. Twelve women were diagnosed as alcohol dependent/ abusers according to criteria established in the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III R). Their daily alcohol consumption ranged from 42-324 grams. Six women were diagnosed as polydrug dependent. In addition to alcohol (84-830 g/day), cocaine was the most frequently abused drug followed by tranquilizers, sedatives, marihuana, amphetamines and opiates. All women received a thorough physical examination and laboratory studies including blood hemogram and chemistry. All patients were drug free and had no signs of withdrawal at the time of sample collection. Blood samples for LH, FSH, prolactin, E_2, progesterone and cortisol were also collected.

Fifty percent of the alcoholic women had hyperprolactinemia (22.3-87.5 ng/ml) independent of amount and duration of alcohol use and 1 had secondary amenorrhea with a normal prolactin level and low levels of LH and E_2. Two polydrug abusers had hyperprolactinemia (25.7 and 29.6 ng/ml) and 1 had secondary amenorrhea (normal prolactin. low LH, FSH and E_2). Hormone levels were consistent with reported menstrual cycle phase or menopausal range in 16 patients. Cortisol levels were also within normal range for all patients. Of the 18 patients, 7 had elevated MCV and 2 were anemic. Four patients had elevated sGPT (67-100 U/L) and 2 had marginally elevated sGOT.

The mechanisms of alcohol and drug induced derangements of reproductive dysfunction have yet to be determined. These data confirm and extend previous reports that alcoholism and drug abuse may be associated with hyperprolactinemia in women. Hyperprolactinemia may cause amenorrhea and

disruptions of menstrual cycle but it is not invariably associated with menstrual cycle abnormalities. The time course and prospects for recovery during abstinence and the extent to which the reproductive system develops tolerance to the disruptive effects of chronic alcohol and drug abuse are unknown. Accumulating evidence of reproductive dysfunction in female alcohol and drug abusers indicates that treatment programs should also evaluate neuroendocrine status.

ACKNOWLEDGEMENTS

This study was supported in part by grants DA 04059, DA 00101, DA 00064 from NIDA and AA 06252 from NIAAA.

AFFILIATION

Alcohol and Drug Abuse Research Center
McLean Hospital
Harvard Medical School
115 Mill Street
Belmont, MA 02178

Comparison of Amantadine and Desipramine Combined with Psychotherapy for Treatment of Cocaine Dependence

W. W. Weddington, B. S. Brown, C. A. Haertzen, J. M. Hess A. F. Kolar and J. R. Mahaffey

This paper reports the results of a single blind, placebo controlled, 12-week trial of amantadine and desipramine for treatment of cocaine dependent outpatients.

METHODS

Subjects. Subjects were consecutively self-referred cocaine addicts who applied for outpatient treatment. Subjects were literate, used a minimum of one gram cocaine per week for 12 weeks prior to treatment, and completed 14 days of initial treatment. Exclusion criteria were current abuse/dependence on any substance other than nicotine, current medical illness, pregnancy, psychosis, or mandated treatment. Subjects were randomly assigned to 1 of 3 treatments: (1) 12 weeks deslpramine 200 mg per day; (2) 4 weeks amantadine 200 mg per day followed by 8 weeks placebo; and (3) 12 weeks placebo.

Services Delivered Subjects attended 3 times per week to receive medication, provide observed urines for toxicology, and received indivldual counseling 2 times per week.

Instruments. Diagnostic assessments were performed using structured questionnaires. Current level of psychiatric symptomatology was determined using the SCL-9Q and the Beck Depression Inventory. Cravlng for cocaine was measured using an analog scale. Blood levels of amantadlne and deslpramine were measured during Weeks 3. 8, and 12.

Data Analysis Results are reported as means +/- SEMs. A one-way analysis of variance for independent measures was used to test differences between groups on demographics and baseline data. A mixed design ANOVA was used to show treatment effects over weeks and the interaction of groups and weeks. To adjust for attrltion, we used the last week of observation carried forward, or "endpoint" analysis.

RESULTS

Sample Characteristics. There were no significant differences between treatment groups regarding demographics, quantity of cocaine used, craving, routes of cocaine administration, and incidence of psychiatric diagnoses.

Outcomes. There were no significant differences between treatment groups regarding weeks in treatment: deslpramlne, 6.8 weeks; amantadine/placebo 7.4 weeks; and placebo, 8.0 weeks (F=.36, df=2/51, p>.05). There were dramatic reductions in cocaine use during treatment in all groups and the lower use persisted throughout treatment (F=26.41, df=12/612, p<.0001), but there were no significant differences in reported cocaine use between the three treatment groups (F=.O8, df=2/51. p>.05). There was a trend toward statistical significance regarding maximum number of weeks of continuous cocaine-free urines: desipramlne, 6.2; amantadine. 3.8, and placebo, 3.6 (F=2.01, df=2/51. p=.13). There were dramatic reductions in craving for cocaine reported by subjects in all treatment groups compared to intake throughout treatment (F=16.20. df=12.612, p<.OOOl), but there were no significant differences among treatment groups (F=0.55. df=2/51, p>.O5). Similarly, there was marked lowering of depression scores by subjects in all groups (F=13.15, df=12/612, p<.0001) but no significant differences among groups (F=.45, df=2/51, p<.O5).

DISCUSSION

Our study demonstrated dramatic decreases in cocaine use and craving for subjects who stayed in treatment. While we observed a trend for subjects receiving desipramlne to maintain a greater number of cocaine-free weeks, we did not demonstrate statistically significant differences among groups for outcome measures. Possibilities for this are that plasma levels were not sufficient; twice-a-week counseling or thrice-weekly urine analyses were sufficiently powerful to overwhelm medication differences; or that amantadlne or deslpramine were not efficacious.

Mean plasma concentration of deslpramine (96.8 ng/ml) in a subsample of our subjects was less than that recommended for treatment of depression (125/ng/ml), thus the dosage of deslpramlne may have been subtherapeutic.

AUTHORS:

W. W. Weddington, B. S. Brown, C. A. Haertzen, J. M. Hess, A. F. Kolar and J. R. Mahaffey
Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland

Combination of Naloxone with Buprenorphine in Humans

Linda L. Weinhold, George E. Bigelow and Kenzie L. Preston

Buptenorphine is a semisynthetic opioid analgesic which has morphine-like agonist effects in non-dependent subjects. The present study was conducted to determine whether simultaneous administration of naloxone anenuates the agonist effects of buprenorphine.

In a resident laboratory, seven nondependent adult male volunteers (28-47 years old), with histories of opioid abuse, participated in within-subject evaluations of the following 7 acute drug conditions: (1) buprenorphine 0.4 mg alone, (2) buprenorphine 0.4 mg plus naloxone 0.4 mg, (3) buprenorphine 0.4 mg plus naloxone 0.8 mg, (4) buptenorphine 0.8 mg alone, (5) buprenorphine 0.8 mg plus naloxone 0.4 mg, (6) buprenorphine 0.8 mg plus naloxone 0.8 mg and (7) placebo. Physiological and subjective measures of acute drug effects were collected at: pre-drug, and 5, 15, 30, 45, 60, 90, 120 min post drug. Data were analyzed by analysis of covariance (ANCOVA) with repeated measures. An overall ANCOVA was performed on all seven drug conditions and additional ANCOVAs were performed on data subsets to determine specific buprenorphine dose effects and naloxone dose effects.

The data analyses indicated that: (1) Bupxenorphine produced dose-related opioid agonist effects on both physiological and subjective indices. (2) The opioid agonist effects of buprenorphine 0.4 mg were themselves mild, but naloxone tended to attenuate those effects on the visual analog High scale, on opioid drug class identification, and observer agonist ratings. (3) With buprenorphine 0.8 mg, which produced more pronounced opioid agonist effects, naloxone significantly attenuated those agonist effects on pupil diameter, respiration rate, and opioid drug class identification. (4) Naloxone, however, did not block all indices of the early buprenorphine agonist effects.

We conclude that a combination product of buprenorphine and naloxone may have lower abuse liability than buprenorphine alone.

Affiliation: Research was conducted at: BPRU. D-5-West, The Johns Hopkins/Key Medical Center, Baltimore, MD 21224. Dr L. L. Weinhold is currently affiliated with NIDA Addiction Research Center, Baltimore, MD.

Acknowledgements: Supported in part by USPHS grants T32 DA07209, R01 DA04089, and K05 DA00050. and by Reckitt and Colman Pharmaceutical Division.

Abuse Liability of Diphenhydramine in Sedative Abusers

Barbara Wolf, John J. Guarino, Kenzie L. Preston and Roland R. Griffiths

Diphenhydramine (Benedryl) , an antihistamine with prominent sedative effects, has been available for more than 30 years. This investigation represents a preliminary assessment of the abuse liability of diphenhydramine (DPH) in 10 subjects with histories of sedative abuse; all subjects reported use of benzodiazepines and 7 subjects had also used barbiturates and/or other sedatives. Subjects were healthy male volunteers ranging in age from 26-44 (mean 33 years). Five subjects were studied on a residential unit (3-5 weeks) and 5 as outpatients (16 days). The study was designed as a single-blind acute dose run-up: initial dose 50-100mg, increments of 50-100mg, maximum planned dose 600mg. Active doses were altered with placebo every other day. The administered maximum dose was 600mg in five, 500mg in two, 400mg in one, and 300mg in two subjects. Five subjects did not receive the maximum dose; in four of them this was due to adverse drug effects such as increase in blood pressure, palpitations, restlessness, derealization (the fifth subject did not receive 600mg for reasons unrelated to the drug effect). Subjects were asked to rate the strength of the drug effect on a 5-point scale from 'not at all' to 'very strong' at hourly intervals after drug administration up to 5 hours; there was a clear dose-related increase in the subject rated peak strength of drug effect up to the maximum dose. DPH produced dose-dependent decrements in two psychomotor performance tasks; balance appeared to be a more sensitive parameter than circular lights. Although DPH was most frequently identified as a barbiturate/other hypnotic or as a benzodiazepine, it was also identified as a stimulant and as an antidepressant. Subjects rated their "liking" on a 5-point scale from 'dislike or feel neutral' to 'very much'; this showed a dose-related increase in peak "liking" up to 600mg in the subgroup of subjects who tolerated these high doses. The "next day liking", estimated the morning after the drug administration essentially covaried with the actual time rated "liking". Similarly, the estimated street value showed a dose-related increase in the subgroup of subjects tolerating 400-600mg. High doses increased subject rated adverse effects such as confusion, forgetfulness, restlessness, anxiety, shakiness, dizziness, dry mouth, tingling, and muscle cramps. These data suggest some abuse potential of DPH in individuals with histories of sedative abuse, but adverse reactions emerging at higher doses may preclude DPH from wide-spread abuse. The finding that DPH may have some abuse liability is supported by clinical data,. i.e., case reports of abuse of cough-syrup by polydrug abusers and the abuse of tripelennamine, another antihistamine, in

combination with pentazocine, the so-called “T's and Blues” (Lahmeyer and Steingold, 1980; Showalter, 1980).
Supported in part by NIDA grant DA-03889.

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Affiliation: The Johns Hopkins University, Francis Scott Key Medical Center, Baltimore, Maryland

Needle-Sharing -Patterns as a Predictor of HIV Seroprevalence Among New York City Intravenous Drug Users (IVDUs)

K. Yee, L. S. Brown, B. J. Primm, T. Nemoto and K. Foster

INTRODUCTION

Although intravenous drug use represents the second most frequent behavior associated with human immunodeficiency virus (HIV) transmission and all AIDS cases, it represents the primary factor in AIDS cases among women, children, ethnic minorities and heterosexually acquired AIDS cases.

Just as previous research studies demonstrated the direct link between certain IV drug using behaviors and an increased risk for HIV infection, an examination of the particular patterns in these behaviors would further enhance our ability to plan effective intervention programs that address the specific needs of IVDUs and their sex partners, especially among ethnic minorities.

This study focused particularly on drug using behaviors, (such as needle-sharing and needle-cleaning patterns, and use of shooting galleries), and their association with HIV seroprevalence among intravenous drug users (IVDUs) in New York City.

METHODOLOGY

Two hundred eighteen newly-admitted patients were recruited from methadone maintenance clinics in Brooklyn and Manhattan, New York in 1987. This sample included 47% Hispanics, 45% blacks, and 8% whites, with a mean age of 33.4 years, of which 64% were males, and 36%. females.

Following informed consent, the patients were administered a standardized questionnaire containing variables pertaining to demographic and socioeconomic status, drug use patterns, and sexual behaviors. Sera were also collected and tested for HIV antibodies via Elisa and Western Blot assays.

Using the SPSS/PC+ statistical software package, unlvarlate and multivariate analyses were used to analyze HIV serostatus by behavioral data, particularly those regarding needle-sharing and needle-cleaning patterns, and shooting gallery visits.

RESULTS

Overall, the HIV infection rate was 60%. The patients who reported more frequent use of shooting galleries were less likely to clean their needles than those who used shooting galleries less often (p=.009). Those who frequented shooting galleries tended to share needles more often than those who did not use frequent them (p=.004). IVDUs who shared needles with the same person for at least one year were at lesser risk for HIV infection (p=.0262), less likely to vist shooting galleries (p=.001), and more likely to be women (p=.03). Also, women were more likely than men to have a spouse who injected drugs (p=.0004). These findings concur with other studies which suggest that women, more often than men, are involved in monogamous relationships, and that this lifestyle usually extends to their drug using behavior.

CONCLUSIONS

The findings of this study suggest that the frequenting of shooting galleries is significantly associated with known risk behaviors such as needle-sharing and a tendency to not clean needles. Therefore, interventions directed at reducing the use of shooting galleries, or at least, modifying behaviors in shooting galleries may reduce the incidences of HIV infection related to IV drug-use. The impact of these interventions may also extend beyond IVDUs and possibly translate into a subsequent decrease in HIV seroprevalence among women, children, and ethnic minorities, along with a decline in the number of cases of heterosexual transmission of HIV.

AFFILIATIONS: Division of Research and Medical Affairs, Addiction Research and Treatment Corporation, Brooklyn, N.Y.; and Harlem Hospital, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, N.Y.

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Effects of Food Deprivation on Subjective Responses to d-Amphetamine and Marijuana in Humans

James Zacny and Harriet de Wit

Two studies examined the effects of 24 hours food deprivation on subjective responses to oral *d*-amphetamine (10 mg) and smoked marijuana (1.3% THC). Both studies used a within-subjects design in which subjects received drug and placebo in both a fed and a fasted state. In both studies, each of the four experimental conditions - FED/DRUG, FED/PLACEBO, FAST/DRUG, FAST/PLACEBO - was enacted twice according to a randomized block design.

In the amphetamine study, 12 normal volunteers received amphetamine or placebo after fasting for 24 hours or while being in a fed state. Three subjective effects questionnaires, the Profile of Mood States, the Addiction Research Inventory, and the Visual Analogue Scale, were completed prior to and 1, 3 and 6 h after the early morning capsule ingestion. Typical elevations in such subjective effects as elation and vigor were obtained after amphetamine ingestion in both feeding conditions, but fasting neither potentiated nor attenuated the drug response. Subjects at the end of the session, however, were more likely in the FAST/DRUG condition than in the FED/DRUG condition to label the capsule they had ingested at the beginning of the session as a stimulant.

In the marijuana study, 8 experienced smokers inhaled six 100-cc boluses of placebo or active marijuana smoke after they had fasted for 24 hours or 1-2 hours after they had consumed a 2000-cal meal. Subjective effects of marijuana were assessed before, during, and after smoking. Smoke inhalation was monitored by measuring expired-air carbon monoxide (CO) levels before and after smoking. CO absorption

from both placebo and active marijuana did not differ across feeding conditions, indicating that smoke dose was similar across feeding conditions. Typical elevations in such subjective effects as “high” and sedation were obtained after smoking active marijuana, but fasting neither potentiated nor attenuated the drug response.

Although these two studies suggest that the subjective effects of drugs are not altered by acute food deprivation, they do not address the issue of whether food deprivation alters the reinforcing effects of psychoactive drugs in humans. Studies which simultaneously assess the subjective and reinforcing effects of drugs as a function of food deprivation would be useful.

Affiliation: Department of Psychiatry, University of Chicago, Chicago, IL

Effects of Acute and Chronic Administration of (+)SKF 10,047 on Body Temperature in the Rat

M. Bejanian, R. N. Pechnick and R. George

INTRODUCTION

Acute administration of PCP induces a dose-dependent hypothermia in rats. In contrast, chronic administration of PCP induces hyperthermia (Pechnick and George, 1989). The characteristics of the expression of the hypothermic and hyperthermic effects of PCP indicate that there could be separate underlying mechanisms for the two effects.

PCP has been shown to interact with two distinct binding sites in the brain. These sites have been termed the PCP and sigma receptors (Quirion. et al., 1987). Although many of the effects of PCP are postulated to be mediated through the PCP receptor, the functional significance of the two receptors in mediating different effects of PCP have not been defined.

Enantiomers of N-allylnormetazocine (SKF 10,047) have been shown to have differential selectivity for sigma versus PCP receptors (Largent. et al., 1986). The purpose of the present study was to characterize the effects of acute and chronic administration of (+)SKF 10,047 (SKF). which has selectivity for sigma over PCP receptors, on body temperature in the rat.

METHODS

Male Sprague-Dawley derived rats were housed individually in a room maintained between 21-22°C. under a 12 hour light-dark cycle (lights on 0700) for one week before testing. On day 1 the rats were weighed and baseline rectal temperatures were recorded. Two additional pre-injection temperatures were recorded at 30 minute intervals. The rats were injected subcutaneously with normal saline or (+)SKF 10,047 (5, 10 or 20 mg/kg). Rectal temperatures were recorded every 30 minutes for 180 minutes post-injection. A second group of rats were tested for the effects of acute administration of 40 and 80 mg/kg SKF on body temperature. Following the pre-injection temperature measurements, the rats were injected with normal saline or SKF (40 or 80 mg/kg) and the temperature time-course followed as described above.

On days 2 to 10, the rats were weighed, rectal temperatures were recorded, after which the tats were injected with saline or the same dose of SKF that they had received on day 1 (5, 10 or 20 mg/kg). On days 7 and 10 full time-course studies were carried out as described above for day 1.

The data were analyzed by analysis of variance. When a significant effect of treatment was found, multiple comparisons were made between treatment groups using Dunnett's r-test or Scheffe's F-Test .

RESULTS

Acute administration of SKF at the doses of 5-40 mg/kg did not have a significant effect on body temperature of the rats. At the dose of 80 mg/kg, SKF produced significant hypothermia. In contrast to day 1. administration of SKF on day 7 to chronically SKF-treated animals produced hyperthermia. The hyperthermic effects of SKF were greater at 20 mg/kg than at 5 and 10 mg/kg. On day 10 of chronic treatment, SKF produced a dose dependent hyperthermia. The hyperthermic effects of SKF were enhanced on day 10 compared to day 7.

Chronic treatment of animals with SKF for 10 days had no significant effect on baseline temperatures and weights. There was a significant effect of time (days of chronic treatment) on body temperature and on weights.

CONCLUSIONS

Acute administration of the selective sigma receptor agonist SKF at doses of 5-40 mg/kg has no significant effect on body temperature in the rat. At the dose of 80 mg/kg, the acute administration of SKF produces significant hypothermia. In contrast, chronic administration of SKF (5-20 mg/kg) produces significant hyperthermia. The hyperthermic response is dependent on the dose and the duration of SKF treatment.

The hypothermic effects of SKF are seen following acute administration of a high dose of SKF (80 mg/kg). In contrast, the hyperthermic effects of SKF are seen following chronic administration at considerably lower doses of SKF. These characteristics indicate that there could be separate mechanisms underlying the two effects of SKF on body temperature.

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ACKNOWLEDGEMENTS

This work was supported by NIDA grants DA-04113 and DA-05448.

AFFILIATION

Department of Pharmacology and Brain Research Institute, School of Medicine, University of California at Los Angeles, Los Angeles, CA 90024-1735.

Effects of Buspirone in the Benzodiazepine Dependent Rat

Norman R. Boisse, Gary M. Samoriski and Yu Xie

The anxiolytic efficacy of buspirone following chronic benzodiazepine use in man is controversial. Benzodiazepine withdrawal may be involved. Accordingly, acute, sub-acute and chronic buspirone experiments were designed to evaluate buspirone (0.1, 1 and 10 mg/kg) effects during maximal benzodiazepine withdrawal and to induce benzodiazepine-like withdrawal in rats. Benzodiazepine dependence was induced with chlordiazepoxide 150 mg/kg b.i.d. x 5 weeks; controls received water; both p.o. Three days post-chlordiazepoxide, when withdrawal is maximal, acute buspirone/water was given p.o. and withdrawal evaluated at 0 and 30 min. by a standard neuro-behavioral screen. In a second chronic chlordiazepoxide/water group, buspirone dosing began 12 hours post-chlordiazepoxide b.i.d. x 3.5 days and withdrawal evaluated. In a third set, rats received chlordiazepoxide 150 mg/kg, water or three buspirone doses b.i.d. x 5 weeks and spontaneous withdrawal evaluated daily x 14 days. Acute and sub-acute buspirone failed to consistently affect benzodiazepine withdrawal. Consistent with clinical reports, buspirone withdrawal also failed to produce a benzo-diazepine-like withdrawal syndrome. Therefore, buspirone is devoid of benzodiazepine-like direct physical dependence potential or cross physical dependence capacity. (Supported by Bristol-Myers grant).

Section of Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115.

Profile of Opioid Withdrawal in Newly Hatched Chickens

Maureen E. Bronson and Sheldon S. Sparber

Interest in the consequences of exposure to opioids during development stems from the fact that many children are born passively addicted to this class of drugs. In the early to mid 1970s, it was not uncommon to read descriptions of morbidity and mortality in such neonates, which in retrospect appears to have been related more to severe abstinence than to opioid exposure *per se*, although the latter may contribute to congenital or postnatal developmental risk factors as well. Unfortunately, in animal research associated with this problem, too little attention has been paid to possible secondary or tertiary causes, such as hypoxia/hypercapnia in pregnant experimental subjects, brought about by inappropriately high doses of the opioid; or treatment protocols which do not model stable maintenance schedules for pregnant women in methadone programs. Additional sources of potential'epiphenomena include nutritional and other neonatal experiences. As a means of eliminating possible maternal factors, our laboratory has used the chicken embryo and neonate to study effects of opioids, opioid withdrawal and quasi-opioid withdrawal during development. The further characterization of withdrawal in the embryo and neonate of this species, and its potential utility for studying the biological bases for the adaptive process (i.e. dependence), its expression (i.e. withdrawal), and the potential utility of selective serotonergic antagonists for nonopioid treatment of withdrawal in the neonate are described in this communication.

The 14-day-old embryo can be shown, under appropriate experimental conditions, to develop acute opioid dependence and express withdrawal (e.g. increased motility) in a manner similar to that observed in older embryos. Moreover, significant changes in brain 5-HIAA concentrations, at a time when dependence can be demonstrated, indicate serotonergic involvement in the adaptive process and its expression.

A single dose of methadone can also produce dependence in the newly hatched chicken, as evidenced by precipitation of withdrawal when naloxone is injected 1-4 hours later. When a low dose of naloxone is used, withdrawal is characterized by head shaking and distress vocalization. Moderate doses of naloxone produce tremor, wing extension and splayed feet, while high doses cause loss of righting reflex, which may be accompanied by convulsive-like movements. If a higher dose of methadone is used to initiate dependence, a much lower dose of naloxone will produce distress vocalization, tremor and wing extension. Opioid dependent human neonates undergoing spontaneous

withdrawal exhibit similar or analogous signs, e.g. high-pitched cry, tremor and, if withdrawal is severe, convulsions (Ostrea, E.M., C.J. Chavez and M.E. Strauss. J. Pediatrics 88:642-645, 1976; Finnegan, L.P. In: Research on the Treatment of Narcotic Addiction, State of the Art. J.R. Cooper, F.Altman, B.S. Brown and D. Czechowica, Eds. DHHS Publication No. ADM 83-1281, Rockville, MD, pp. 392-443, 1983).

We were also able to demonstrate dose-dependent quasi-opioid withdrawal in the neonatal chicken. The syndrome produced by 10 mg IBMX/kg was exacerbated by a low dose of naloxone. but the greater behavioral syndrome produced by 20 mg IBMX/kg was not. We have recently reported this same phenomenon in the rat (Kleven. M.K. and S.B. Sparber. J. Pharm. Exp. Ther. 248:273-277, 1989).

Thus, the chick embryo and/or neonate provides a good model for studying the consequences of either opioid dependence and withdrawal or withdrawal alone (IBMX) during early development; and it should also provide a basis for predicting potential nonopioid treatment modalities.

In support of this notion, we have recently observed that the 5-HT_2 antagonist mianserin. which attenuates true and quasi-opioid withdrawal in mature rats (Neal, B.S. and S.B. Sparber, J. Pharm. Exp. Ther. 236:157-165, 1986; Kleven, M.K. and S.B. Sparber, Psychopharmacology, in press), also blocks the long term learning impairment and altered reactivity to morphine in rats given IBMX as infants if mianserin is coadministered with the IBMX (Neal, B.S. and S.B. Sparber, Xth International Congress of Pharmacology, Syndey, Australia, 1987). We now report that mianserin produces dose-dependent attenuation of distress vocalization in l-day-old chicks undergoing acute withdrawal from methadone plus naloxone. Significant effects were seen after pretreatment with 1 mg mianserin/kg and vocalization was not different from controls after 5 mg mianserin/kg. This finding, combined with the evidence of serotonergic involvement in the adaptive process of opioid dependence in the embryo, suggest that selective serotonin receptor antagonists may prove useful for detoxification during early development. Supported by USPHS grant DA01880 and T32 DA 07097.

AUTHORS:

Maureen E. Bronson, Ph.D.
Sheldon S. Sparber, Ph.D.
Department of Pharmacology
University of Minnesota
Minneapolis, Minnesota 55406

Synthesis of Fentanyl Analogs

F. Ivy Carroll, Karl G. Boldt, P.-T. Huang, D. K. Sawyer and George A. Brine

There are several classes of synthetic piperidine derivatives which show analgesic activity, the 4-anilinopiperidine structure (1, fentanyl class) having provided the most potent compounds. An enormous number of fentanyl analogs has been prepared, many of which are substantially more potent than morphine. A large number of fentanyl analogs has obtained clinical utility. The fentanyl class of analgesics has also been an attractive target for "designer drug" chemists. Beginning in 1981 when street samples of "China White" were identified as α-methylfentanyl (Kram et al., 1981), an increasing number of fentanyl analogs has been identified as "designer drugs". The efforts to curb the abuse of fentanyl analogs have required authentic compounds as reference standards for abuse liability studies. We have synthesized fentanyl (1a) and the twenty analogs summarized in the Chart by adaptation of reported, syntheses (Bever et al., 1974; Casey et al., 1969; Daele et al., 1976; Fifer et al., 1984; Janczyk et al., 1978; Janssen, 1965; Lobbezoo et al., 1980; Lobbezoo et al, 1981; Maryanoff et al., 1982; Zee et al., 1981; Zhu et al., 1983). All of the compounds were purified as the hydrochloride salts and characterized by TLC, melting point, ^{1}H NMR, IR and elemental analysis as necessary. To our knowledge, compounds 1f-h, 2 and 6b have not been previously reported in the literature.

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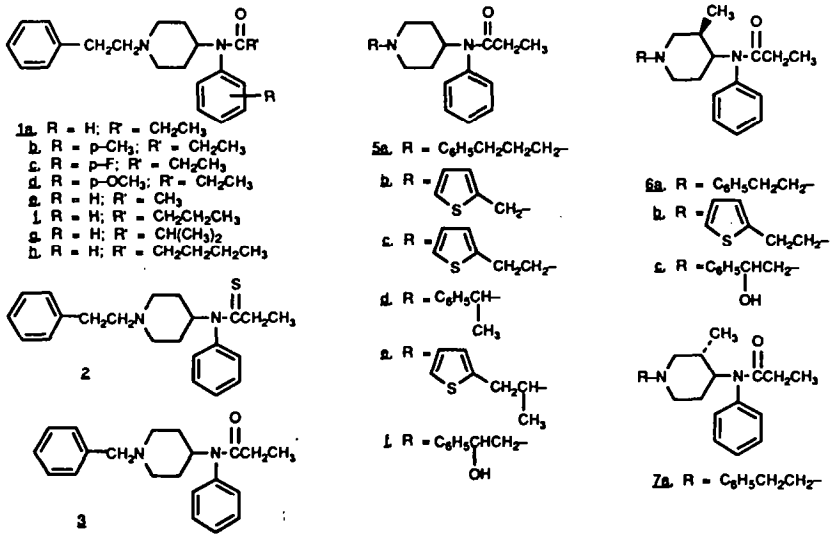
AFFILIATION

Chemistry and Life Sciences, Research Trlangle Institute, Research Trlangle Park, North Carolina 27709, USA.

Chart



Effects of D1 and D2 Dopamine Antagonists on Heroin-Trained Drug Discrimination

William A. Corrigall and Kathleen M. Coen

Rats were trained to discriminate heroin (0.3 mg/kg from saline in a fixed-ratio 10 food-reinforced paradigm. The discriminative stimulus produced by heroin was first characterized in tests with heroin metabolites monoacetyl morphine (MAM) and morphine (M); MAM appears to be responsible for the heroin-trained stimulus, since equi-molar doses of heroin and MAM were similar. In contrast, a dose of 3 mg/kg M was the lowest dose that generalized completely to the heroin-trained stimulus. At the training dose of heroin, doses of the D1 antagonist SCH23390 and the D2 antagonist spiperone between 0.001 and 0.01 mg/kg did not alter responding on the drug-trained lever or response rate. At doses of either D1 or D2 antagonist of 0.03 mg/kg and higher, both drug-trained responding and response rate decreased in parallel. The antagonists were also ineffective in altering the heroin stimulus at lower heroin doses. These results suggest that the discriminative stimulus properties of heroin do not depend significantly on dopaminergic function.

AUTHORS:

William A. Corrigall
Kathleen M. Coen
Addiction Research Foundation
Toronto, Ontario, Canada M5S 2S1

The Stimulus Properties of Cocaine:. Effects of Bay K 8644 and Nimodipine

Kathryn A. Cunningham and Patrick M. Callahan

As a result of the epidemic abuse of cocaine in the United States and the increased incidence of adverse effects associated with cocaine exposure, attempts to both understand its mechanism(s) of action and explore pharmaceutical compounds which may be useful in the treatment of cocaine abusers are underway. The dihydropyridine calcium channel blockers may be of use in this regard as nifendipine has recently been shown to antagonize the cardiac toxicity and lethal effects of cocaine (Trouve and Nahas, 1986). Nifendipine and other dihydropyridines readily penetrate the blood brain barrier (Schoemaker et al., 1983), appear to bind to discrete areas of the brain (Gould et al., 1985) and are reported to antagonize some stimulant-induced behaviors (Fung and Uresky, 1980; Grebb, 1986). The present experiment was designed to investigate whether nimodipine might block the subjective effects of cocaine. We have also assessed whether the calcium channel agonist BAY K 8644 mimics the interoceptive cocaine cue.

METHODS

Male Sprague-Dawley rats (N=8) were trained to discriminate cocaine (10 mg/kg) from saline in a two-choice, water-reinforced (FR 20) drug discrimination task using procedures that have been described in detail elsewhere (Cunningham and Appel, 1987). After acquisition of the discrimination and establishment of the dose-response relationship, substitution tests with BAY K 8644 administered either intraperitoneally (IP; 15 min) or orally (PO; 30 min) in an ethanol/polyethylene glycol solvent (provided by the supplier) and combination tests with nimodipine (IP; 60 min; dissolved in a similar solvent) were conducted.

RESULTS

Cocaine (0.625-10 mg/kg) produced a dose-related increase in responding on the drug-lever. In substitution tests, BAY K 8644 when administered either intraperitoneally (0.25-1.0 mg/kg; IP) or orally (1 or 2 mg/kg; PO) engendered predominantly saline-lever responding. When given in combination with cocaine (0.625-10 mg/kg), nimodipine (0.2 or 0.4 mg/kg) tended to shift the dose-response curve to the right. Nimodipine (0.8 mg/kg) also appeared to reduce cocaine-lever responding after lower cocaine doses (1.25 and 2.5 mg/kg). However, drug-lever responding did not differ significantly for any combination of cocaine plus nimodipine. The

percent cocaine-lever responding at 5.0 and 10 mg/kg was unaffected by the highest dose of nimodipine tested (1.6 mg/kg), while lower cocaine doses in combination with nimodipine (1.6 mg/kg) could not be tested because of response depression. Neither vehicle nor nimodipine administered alone elicited responding on the drug lever.

DISCUSSION

The calcium antagonist BAY K 8644 crosses the blood-brain barrier, alters central catecholamine synthesis and release (Pileblad and Carlsson, 1987; Woodward and Leslie, 1986) and elicits significant behavioral effects (Bourson et al., 1989; O'Neill and Boiger, 1988). In fact, BAY K 8644 is discriminable from saline and this cue is antagonized by nifendipine (Gladstein et al., 1987). In the present study, the subjective state induced by BAY K 8644 is not perceived as similar to that induced by cocaine.

Nimodipine only partially blocks the subjective effects of cocaine, but this antagonism is not robust nor dose-related. Our finding corroborates those of Nencini and Wooiverton (1988) who reported similar attenuating effects of 2 mg/kg, but not higher doses (4.0 and 5.6 mg/kg), of nimodipine. As suggested by Nencini and Woolverton (1988), the partial attenuation of stimulant cues may be related to non-specific stimulus factors elicited by the combination of amphetamine (or cocaine) with the calcium channel antagonist.

While calcium is a ubiquitous ion important in many biological functions, including neurotransmitter release, our findings with BAY K 8644 and nimodipine suggest only a subtle influence of this mediator in the subjective state induced by cocaine.

Supported by Miles Institute for Preclinical Pharmacology.

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Indomethacin Antagonizes the Effects of Ethanol: Effect of Route of Administration

G. I. Elmer and F. R. George

INTRODUCTION Ethanol is proposed to initiate some of its biochemical and behavioral effects via fluidization of neuronal membranes (Goldstein et al., 1982). Since the arachidonic acid cascade is significantly influenced by changes in lipid fluidity, the behavioral effects of ethanol have been proposed to be due in part to an increase in arachidonic acid metabolites. In support of this hypothesis, prostaglandin synthetase inhibitors (PGSI) have been demonstrated to be effective antagonists of the acute activating, hypothermic, hypnotic and lethal effects of ethanol (George, 1989). The ability of prostaglandin synthetase inhibitors to antagonize the effects of ethanol, however, has been demonstrated only via the intra-peritoneal (i.p.) route of administration. The pharmacological effects of these inhibitors could be due in part to the inhibition of the local release of prostaglandins caused by the irritant effects of i.p. ethanol. Therefore, the effect of route of administration on the ability of the potent PGSI, indomethacin, to inhibit the acute hypnotic effects of ethanol was investigated. In addition to delineating the CNS versus peripheral effects of indomethacin, the clinical utility of prostaglandin synthetase inhibition in treating acute oral ethanol hypnosis will be determined.

METHODS The effect of administration route was investigated as a function of indomethacin dose. Indomethacin (0-10 mg/kg) was given to male DBA/2J mice 15 minutes post 4.0 g/kg ethanol treatment. Route of administration was varied in the following order: i.p. ethanol (50% v/v) and i.p. indomethacin; i.g. ethanol (20% v/v) and i.p. indomethacin; i.g. ethanol (20% v/v) and i.g. indomethacin. The duration of loss of the righting reflex following ethanol administration was used as the measurement for the acute hypnotic effects of ethanol. After the mice were able to right themselves three times within 30 seconds, duplicate 10 ul retro-orbital blood samples were obtained and analyzed for blood ethanol concentration. A minimum of 5 subjects were used per indomethacin dose and route of administration.

RESULTS Indomethacin significantly antagonized the acute hypnotic effects of ethanol across all routes of administration.

(F(indomethacin)=3.38; df=4,108; p .05). For example, indomethacin at 2.5 g/kg decreased ethanol-induced loss of the righting reflex 31%, 48%, and 49% when administered under the following conditions; (i.g. ethanol/i.g. indomethacin), (i.g. ethanol/i.p. indomethacin) and (i.p. ethanol/i.p. indomethacin), respectively. Route of administration significantly affected indomethacin dose-response curve, (F(route)=3.55; df=2,108; p .05). Indomethacin was most efficacious following i.p. ethanol administration; sleeptime was decreased by greater than 60% versus 38% and 33% for the i.p./i.g. and i.g./i.g. conditions, respectively. Under all conditions, indomethacin treated mice regained the righting reflex at higher blood ethanol levels than did control mice.

DISCUSSION Inhibition of prostaglandin synthetase activity and the subsequent production of prostaglandins antagonizes the acute effects of ethanol across all routes of administration tested. The demonstration that indomethacin is an effective ethanol antagonist given i.p. or i.g. following i.g. ethanol administration support a CNS versus peripheral site of action. That is, variation in site of administration for ethanol and indomethacin decreases the likelyhood that indomethacin inhibited locally released eicosanoids as the primary explanation for its ability to antagonize the effects of ethanol. In addition, indomethacin treated animals regained the righting reflex at significantly higher blood ethanol levels thereby supporting a decrease in neurosensitivity to ethanol as a result of prostaglandin synthesis inhibition. These results combined with the previous demonstration of indomethacins ability to antagonize the acute hypnotic and lethal effects of ethanol extend the range of conditions over which indomethacin is effective in antagonizing the effects of ethanol. Importantly, these results demonstrate the application of prostaglandin synthesis inhibition to clinically relevant orally administered ethanol conditions.

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AFFILIATIONS: NIDA/Addiction Research Center, Behavior Genetics Laboratory, Preclinical Branch, Baltimore, MD, 21224. University of Maryland at Baltimore, Department of Pharmacology and Toxicology, Baltimore MD, 21222.

Potential Neurotoxic Effects of Self-Administered Cocaine on Dopamine Receptors

Nick E. Goeders, Marcia A McNulty, Ann M. Guidroz and Steven I. Dworkin

INTRODUCTION

Until recently, cocaine was viewed by the general population to be a relatively benign substance. Current evaluations of the neurobehavioral effects of the drug have indicated that the term “benign” is a very inappropriate misnomer. Cocaine is one of the most efficacious reinforcers available, and while cocaine use has continued to increase over the last ten years, the use of other behaviorally active drugs has steadily declined. However, the neurotoxic effects of cocaine have received little attention even though the effects of the drug in kindling seizures and the neurotoxic effects of similar drugs have been demonstrated. While the effects of cocaine on neurotransmission are extremely complex, a variety of clinical and experimental data suggest that the behavioral actions of the drug are mediated at least in part through an inhibition of dopamine uptake into presynaptic nerve endings. These experiments were therefore designed to investigate the potential neurotoxic effects of self-administered cocaine on pre- and postsynaptic dopaminergic receptors in the rat brain using light microscopic quantitative autoradiography.

METHODS

Groups of three adult male Fisher 344 littermate rats were used in these experiments. Briefly, the self-administration littermate was trained to respond under a fixed-ratio 2 schedule of cocaine presentation (0.33 mg/200 µl infusion) during daily 6 hour sessions. The yoked-cocaine and yoked-saline littermates received simultaneous infusions of cocaine or saline, respectively, each time that the drug was self-administered by the first littermate. After a 30 day exposure to the drug, the animals were anesthetized with sodium pentobarbital and were sacrificed by cardiac perfusion. The brains were rapidly removed, embedded in brain paste and frozen onto microtome chucks over dry-ice. Ten micron coronal sections were cut in a cryostat microtome, and the sections were thaw-mounted onto subbed slides and stored at -20°C until needed.

Postsynaptic D_2 dopaminergic receptors were labeled under standard conditions using [^{3}H]sulpiride. [^{3}H]Mazindol was used in the presence and absence of desmethylimipramine to label noradrenergic (desmethylimipramine-sensitive) and dopaminergic (desmethylimipramine-insensitive) uptake sites in adjacent tissue sections. After incubation, the slides were dried, affixed to mounting board, placed in x-ray cassettes with radioactive standards and apposed to [^{3}H]Ultrofilm. After a 4 to 6 week exposure, the films were developed using standard procedures and the density of silver grains on [^{3}H]Ultrofilm was quantified using computer-assisted microdensitometry.

RESULTS

Both contingent and non-contingent cocaine administration resulted in decreases in specific [^{3}H]sulpiride binding in the caudate nucleus when compared to yoked-saline control animals, although this effect was statistically significant only in the self-administration rats ($p < 0.05$). There were no statistical differences in specific binding in the nucleus accumbens. Both contingent and non-contingent cocaine administration resulted in statistically significant increases ($p < 0.05$) in desmethylimipramine-insensitive [^{3}H]mazindol binding in both the caudate nucleus and nucleus accumbens when compared to the yoked-saline control animals.

CONCLUSIONS

Both contingent and non-contingent cocaine administration resulted in an increase in dopaminergic uptake sites in the caudate nucleus and nucleus accumbens. On the other hand, cocaine administration resulted in a decrease in postsynaptic D_2 dopaminergic receptors in the caudate nucleus, although this effect was statistically significant only in the self-administration animals. These data are consistent with current hypotheses concerning the neurochemical actions of cocaine. If cocaine binds to presynaptic uptake sites to inhibit the reuptake of dopamine, then an increase in these uptake sites would be expected. The increased synaptic concentration of dopamine would then result in the compensatory decrease in postsynaptic dopaminergic receptors observed in these experiments. These neuroadaptations may be reflective of potential neurotoxic effects resulting from cocaine administration. Additional studies are in progress to further characterize these potentially neurotoxic effects. (Research supported by NIDA Contract # 271-87-8118).

Departments of Pharmacology & Therapeutics and Psychiatry
Louisiana State University Medical Center
Shreveport, LA 71130

Assessment of the Abuse Potential of Methocarbamol in Primates

Belinda A. Hayes and Robert L. Balster

The abuse potential of the muscle relaxant methocarbamol was evaluated in rhesus monkeys. In one study, the ability of i.v. methocarbamol to serve as a reinforcer was evaluated using a standard substitution procedure. In another study, the barbituate-like discriminative stimulus effects of methocarbamol were studied in monkeys trained to discriminate pentobarbital (8.0 mg/kg) from no-drug. Methocarbamol failed to function as a positive reinforcer when cocaine (0.033 mg/kg/injection) or methohexital (0.1 mg/kg/injection) was the baseline drug. when methocarbamol (3.0 and 10.0 mg/kg/injection) was substituted for the two baseline drugs, rates of self-administration fell within saline and methocarbamol vehicle ranges. In contrast, pentobarbital (0.25 and 0.5 mg/kg/injection) provided evidence for reinforcing effects when substituted for methohexital, although not when substituted for cocaine. Methyocarbamol (3-75 mg/kg) failed to generalize from the training dose of pentobarbital (8.0 mg/kg) and also failed to affect overall rates of responding. These results indicate that methocarbamol does not possess reinforcing effects or pentobarbital-like discriminative effects in primates over the dose range tested.

AFFILIATION

Department of Pharmamlogy & Toxicology, Medical College of Virginia, Virginia Ccmmonwealth university, Richmond, Virginia 23298-0613.

Studies on the Stereoselective Synthesis of cis-3-Methyfentanyl

Fu-Lian Hsu, Peter W. Von Ostwalden, Harold D. Banks and C. Parker Ferguson

Fentanyl **(1)** was synthesized by Janssen and his co-workers in 1964 and was one of the most potent synthetic opioids developed. In the tail-withdrawal test in rats, it was found to have almost 200 times the potency of morphine. Its onset time and duration of action are much shorter than that of morphine. In 1973, Riley et al. synthesized 3-methylfentanyl **(2)** from 4-anilino-3-methylpyridine. Shortly thereafter, Janssen's group published a more practical method of synthesizing **2** from 3-methyl-4-piperidone, which is analogous to their fentanyl synthesis. The Schiff base reduction with $NaBH_4$, obtained 70% *cis* and *30% trans* of **5** which was then separated. The *cis*- and *trans*-**5** were then successfully converted to *cis*- and *trans*-3-methyl-fentanyl in 3 steps. In the tail-withdrawal test, the racemic *cis*-**2** was found to be 8 times more active than the trans-isomer, which was equipotent to fentanyl. The *cis*-**2** was then resolved. The ED_{50} of (+)-*cis*-3-methylfentanyl was found to be 20 times lower than fentanyl and was 100 times more potent than the (-)-*cis*-3-methylfentanyl. In view of the high potency of the *cis*-isomer of substituted fentanyls, we became interested in developing an efficient methods for their preparation.

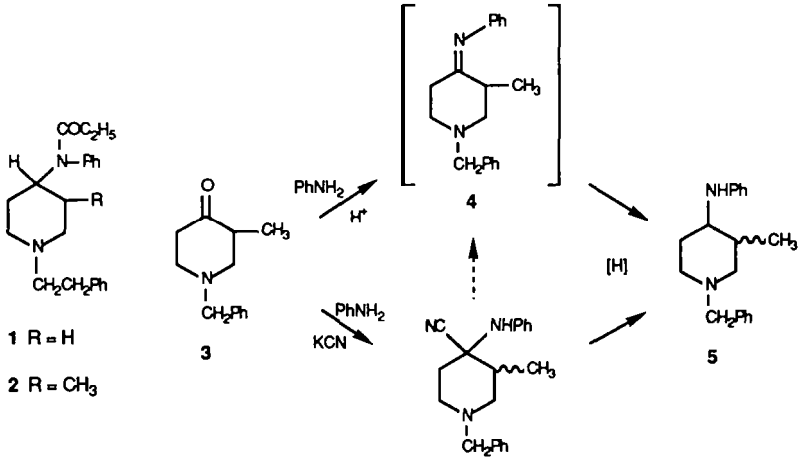
To study the stereoselective synthesis of *cis*-3-methylfentanyl, we developed two different approaches: A) bulky hydride reduction of the Schiff base, **4;** B) reductive decyanation of the α·-aminonitrile, **6.**

To study the stereoselective reduction of the Schiff base, several bulky hydride reducing agents were employed: Super-Hydride, Red-Al, and L-Selectride. The known geometric isomers, *cis*- and *trans*-**5,** were used as the reference for comparison. The relative ratios of the *cis*- and trans-isomers formed by hydride reduction of **4,** were determined by gas chromatography of the crude reaction products. The results showed that a distinctly greater stereoselectivity in the case of L-Selectride which formed a -14-15 fold excess of the *cis*-isomer. When the reduction of **4** with L-Selectride was scaled up, the major *cis*-**5** was obtained in 45% yield as oxalate salt without a tedious

separation of the isomers and was more convenient than the literature procedure (39%).

The reductive decyanation of a-aminonitriles with $NaBH_4$ is well-known. In the model study, the reduction of an analog of **6** (without 3-methyl group) with $NaBH_4$ or other bulky hydride reducing agents, such as Red-Al, Super-Hydride, and L-Selectride gave a clean conversion to the decyanated product. In view of the possibility of the stereoselective decyanation of **6,** with these bulky hydride reducing agents and the elucidation of the mechanism of decyanation in this system, the *cis*- and *trans*-a-aminonitrile, **6** were used for this study. In the reduction of *cis*- and *trans*-**6,** the distributions of *cis*- and *trans*-**5** were similar which was almost identical to the direct Schiff base reduction. As in the case of the Schiff base reduction, L-Selectride was the most stereoselective reagent. It is reasonable to conclude, therefore, that the mechanism of the reaction of *cis*- and *trans*-**6** probably proceeds through a common Schiff base intermediate.

From these studies, it was found that L-Selectride was by far the most stereoselective reagent of the metal hydrides used in both the Schiff base reduction and the reductive decyanations of a-aminonitrile, producing more than a 13-fold excess of the pharmacologically more active cis-isomer.



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[1] U.S. Army Chemical Research, Development & Engineering Center, Aberdeen Proving Ground, MD 21010-5423 and [2]Youngstown State University, Youngstown, OH 44555

Energy Substrate Metabolism in Testis of Rats Treated with Delta-9-Tetrahydrocannabinol (THC) and Cocaine (COC)

Syed Husain

Utilization of substrates like glucose and fructose is an important source of energy for the normal functioning of different tissues in the body. This is particularly true in case of brain and testis where the demand of these substrates is much greater.

Marihuana and its psychoactive constituent Δ^9-THC (THC) have been shown to inhibit different gonadal functions. We suggested that THC causes these reproductive insults by inhibiting the utilization of energy producing substrates in the testis. More recently, the abuse of cocaine (COC) has also become widespread in the younger population of the society. Although the primary neurochemical actions and reinforcing properties of COC have been studied extensively, not much information is available as to the effects of COC on energy metabolism in the testis. Therefore, the following studies were conducted to compare the effects of THC and COC on the metabolism of glucose in the rat testis.

Groups of rats were treated acutely with 10mg/kg,po,THC or 20mg/kg,ip,COC. Control rats received 2ml/kg of sesame oil or saline. THC treated rats were sacrificed 2hr post injection whereas COC animals were killed at 15 or 30 min following drug injection. Their testes were rapidly removed and sectioned into small pieces. With these tissues, radio-respirometric experiments were conducted using 5.5mM radiolabled glucose as the substrate. In these experiments, the rate of $^{14}CO_2$ production was used as an index of glucose metabolism in these tissues. These rates in $^{14}CO_2$ production from testes of THC and COC treated rats were compared with respective controls to determine their significance.

Previously, our studies have indicated that in-vitro as well as in-vivo exposure of rat testicular tissue

to THC causes a significant decrease in the utilization of energy rich substrates by this tissue. Others reported a consistant correlation between the inhibition of fructose utilization and a decrease in the motility of sperms from human, rhesus monkey and rabbits exposed to THC. Similarly, glucose was found to be a necessary substrate for in-vitro capacitation and acrosomal reaction of hamster sperms. These observations indicate an important role for glucose and fructose metabolism in explaining different gonadal effects of THC in the testis.

In recent years, the street use of COC has increased considerably. Studies have shown that behavioral and functional modifications after COC use are related to blockade of reuptake *of* both dopamine and norepinephrine. Similarly, the increased locomotor activity following COC is correlated with an increase in glucose utilization in medial frontal cortex and nucleus accumbens of the mesocorticolimbic system. As metabolism of energy rich substrates like glucose is also vital to the functioning of tissue like testis, we compared the effects of THC and COC on glucose metabolism in this tissue. In this study a 10mg/kg, po dose of THC caused a reduction of 29% in glucose metabolism as compared to controls ($p<0.001$). On the other hand, testicular tissue of rats treated with 20mg/kg, ip COC and sacrificed either at 15 or 30 min had no significant difference in glucose metabolism from their respective controls. These observations suggested that as oppose to THC, COC may have no effects on energy metabolism in the testis. However, recent-reports indicate that effects of COC in humans as well as in other animals vary both with dose and with the route of its administration. In these investigations, intravenous (iv) administration of COC but not the intraperitoneal (ip) route resulted in significant increases in the rate of glucose use in medial prefrontal cortex and nucleus accumbens. In our study, we administered COC by the ip route. This may have contributed to the lack of COC effects on glucose metabolism in the testis. It is likely that following ip administration, the time required for COC absorption is long enough for plasma esterases to degrade the drug to its inactive metabolites. On the other hand, iv administration,may allow enough -active drug to reach the target tissue before it is metabolized (supported in part by NIDA grants DA035595 and DA05215).

AFFILIATION

Department of Pharmacology, School of Medicine, University of North Dakota, Grand Forks, ND 58201

MK-801 Attenuates the Methamphetamine Induced Decreased in Tryptophan Hydroxylase Activity

J. W. Gibb, Michel Johnson and G. R. Hanson

Methamphetamine (METH) decreases the activity of brain tryptophan hydroxylase (TPH) as well as the concentrations of 5-hydroxytryptamine and 5-hydroxindoleacetic acid. Since this alteration of serotonergic parameters persists for long periods of time, it is suggested that the neurochemical changes are accompanied by neuronal degeneration. Because N-methyl-D-asparate (NMDA)receptors have been implicated in ischemia-induced neuronal damage (Simon et al., Science 226:850, 1984), in this study we examined whether blockade of NMOA receptors by an NMDA antagonist attenuates the decrease in TPH activity induced by METH. Male Sprague-Dawley rats (180-250 g) received METH (4 x 15 mg/kg, at 6-h intervals). Rats were sacrificed 18-20 h after the last dose, the brain was removed and the neostriatum and frontal cortex were excised.

METH decreased neostriatal TPH activity to 26% of control. When the non-competitive NMDA receptor antagonist, MK-801 (2.5 mg/kg), was administered 15 min prior to each METH administration, the METH-induced effects on the TPH activity was depressed to only 66% of control. METH decreased cortical TPH activity to 25% of control; when MK-801 was combined with METH administration, TPH activity was 61% of control. When MK-801 was administered alone, there was no effect on TPH activity.

3,4-Methylenedioxymethamphetamine (MDMA, 10 mg/kg), an amphetamine analog, also decreased TPH activity in the neostriatum to 34% and 30% of control in the neostriatum and frontal cortex, respectively. In contrast to METH when MK-801 was administered in combination with MDMA, no effect of the MK-801 was observed. This study suggests that glutamate may play a role in the METH-induced decline in central TPH activity; however the mechanism by which MDMA decreases TPH activity may differ from that by which METH causes its effects.
(Supported by USPHS grants DA 00869 and DA 04221).

AFFILIATION:
Department of Pharmacology and Toxicology
University of Utah
Salt Lake City, Utah 84108

Cocaine Increases Benzodiazepine Receptors Labeled in the Mouse Brain In Vivo with [^{3}H]Ro 15-1788

Marianna E. Jung, Marcia A. McNulty and Nick E. Goeders

INTRODUCTION

The non-medical use of cocaine has continued to increase during the last decade resulting in considerable interest in the clinical and neurobiological implications of this behavior. In humans, benzodiazepines are often employed clinically for the medical complications associated with acute cocaine toxicity and to manage symptoms associated with withdrawal. Convulsions are often manifested as a major side effect of acute cocaine intoxication, and these seizures can be treated with intravenous diazepam, but not dilantin. In addition, major symptoms associated with cocaine withdrawal include severe agitation, anxiety and restlessness. Although diazepam can be used to treat cocaine-induced anxiety, benzodiazepines are not recommended as the treatment of first choice for cocaine withdrawal since the use of these drugs could result in a secondary dependence. Nevertheless, we have reported previously that cocaine administration results in significant changes in the *in vitro* distribution of benzodiazepine receptors measured in discrete regions of the rat brain. The following experiments were therefore designed to investigate the acute effects of cocaine on benzodiazepine receptors measured *in vivo* using [^{3}H]Ro 15-1788.

METHODS

Restrained adult male ICR mice (30 to 35 grams) were injected via the tail vein with 167 µCi/kg (5 µCi/mouse) of [^{3}H]Ro 15-1788 (79.8 Ci/mmol; NEN) diluted with 0.9% saline. to 33 µCi/ml. For determinations of nonspecific binding, mice were pretreated with clonazepam (5 mg/kg, ip) 30 minutes before the intravenous injection. To determine the effects of cocaine on benzodiazepine receptor labeling *in vivo,* various doses of the drug (5, 20 or 40 mg/kg, ip) were administered 5 minutes before the intravenous injection. This time point was chosen so that the maximum effects of the drug would be present at the time of sacrifice (15 minutes after the cocaine injection). Sulpiride (5 mg/kg, ip) was injected 30 minutes prior to the intravenous injection of [^{3}H]Ro 15-1788 to determine

whether the effects of cocaine were mediated via a dopaminergic mechanism. The mice were sacrificed 10 minutes following the intravenous injection, and the brains were rapidly removed and dissected over ice. Samples of the cerebral cortex, hippocampus, diencephalon, cerebellum, striatum and brain stem were retained, weighed and placed in glass scintillation counting vials, and the tissue was allowed to dissolve for 24 hr at 40°C. After the digestion of the tissue was complete, radioactivity was determined using liquid scintillation spectrophotometry.

RESULTS

Cocaine pretreatment (20 mg/kg, ip) resulted in region-specific increases in binding, with statistically significant increases observed only in the hippocampus, diencephalon and striatum ($p < 0.05$). No effects on nonspecific binding were seen in any brain region with any dose of cocaine or following pretreatment with sulpiride. Low doses of cocaine (5 mg/kg, ip) resulted in small but nonsignificant increases in specific binding in all brain regions studied. Higher doses (20 mg/kg, ip) resulted in site-specific increases, while doses just below the convulsant range (40 mg/kg, ip) resulted in significant increases in [^{3}H]Ro 15-1788 binding all brain regions. Sulpiride alone (5 mg/kg, ip) resulted in significant increases in specific [^{3}H]Ro 15-1788 binding in most brain areas studied. However, sulpiride pretreatment did not result in further increases in the effects of cocaine (20 mg/kg, ip) on [^{3}H]Ro 15-1788 binding in any brain region except for brain stem.

CONCLUSIONS

Cocaine pretreatment (20 mg/kg, ip) resulted in region-specific increases in benzodiazepine receptors labeled *in vivo* with [^{3}H]Ro 15-1788. Receptors in these same brain areas are also affected by acute and chronic cocaine administration *in vitro.* These cocaine-induced site-specific increases in binding were also dose-related. Wiih higher doses (40 mg/kg, ip), cocaine resulted in increases in specific binding in all brain regions studied, an effect similar to that produced by buspirone or neuroleptic drugs. The mechanism(s) of action mediating these effects is unclear. However, the cocaine-induced increases in benzodiazepine receptor labeling probably did not result from a direct dopamine-related action since the blockade of D_2 dopaminergic receptors with sulpiride did not alter the effects of cocaine on [^{3}H]Ro 15-1788 binding *in vivo* Additional experiments are in progress to explore the potential mechanisms mediating these effects. (Research supported by USPHS Grant DA 04293).

Departments of Pharmacology & Therapeutics and Psychiatry
Louisiana State University Medical Center
Shreveport, LA 71130

Structural Requirements for Nicotinic Antagonists in the CNS

Thomas J. Martin, John Suchocki, Everette L. May and Billy R. Martin

Nicotinic antagonists have been instrumental in the characterization of nicotine's pharmacology. . Initial evidence for the existence of central nicotinic receptors was based on the fact that nicotine: exerted numerous behavioral effects that were antagonized by ganglionic blockers that penetrated the blood-brain barrier. The mechanism of action of nicotinic antagonists has been studied most intensively in the periphery. These compounds have not been shown to compete effectively for agonist binding sites in the CNS, suggestin that their binding site is distinct from that of nicotine. A binding site for these compounds has yet to be elucidated, however. One criterion for a receptor-mediated mechanism of action is structural requirements for activity Although the structural requirements for antagonism of nicotine has been studied extensively in the periphery, the structural requirements for antagonism of nicotine's behavioral effects has yet to be thoroughly documented.

The antagonism of nicotine-induced antinociception and depression of spontaneous activity was studied in mice for a variety of mecamylamine (N, 2, 3, 3-tetramethyl-2-norbornamine) analogs. All compounds were injected s.c. 10 min prior to s.c. administration of an ED_{84} dose of nicotine. Antinociception was assessed by the tail-flick method 5 min following administration of nicotine or saline. Spontaneous activity was measured by interruptions of infrared beams for a 10 min session 10 min following nicotine or saline administration. Nicotine produced antinociception with an ED_{84} of 2.56 mg/kg. The AD_{50}'s (95% C. L.) of pempidine, (±)-exo-mecamylamine, (-)-exo-mecamylamine, (+)-exo-mecamylamine and (±)-endo-2-desmethylmecamylamine were 0.13 (0.05-0.29), 0.08 (0.02-0.29), 0.09 (0.04-0.23), 0.24 (0.10-0.57) and 0.13 (0.07-0.27), respectively. Endo- and exo-2-norbornamine, as well as their N-methylated analogs, produced a non-dose-responsive antagonism of no greater than 50% at

doses up to 10 mg/kg. Several N-alkylpyridine-substituted analogs of 2-norbornamine produced similar effects. Translocation of the gem-dimethyls to the methylene bride significantly reduced the activity of mecamylamine 4.5 fold, and the N-dimethyl analog of this compound was 9 times less potent than mecamylamine.

Nicotine depressed spontaneous locomotor activity with an ED_{84} of 1.71 mg/kg. When the abovementioned analogs were evaluated for their ability to prevent nicotine-induced hypoactivity, a structure-activity relationship was obtained which was similar to that described for blockade of nicotine-induced antinociception.

Therefore, mecamylamine antagonizes the behavioral effects of nicotine with modest stereoselectivity ((+)-exo/(-)-exo= 2.7). Furthermore, the methyl groups in the N-, 2-, and 3-positions appear to be necessary for optimal antagonistic activity. These structural requirements are distinct from those that have been reported for nicotinic agonists in the CNS, suggesting that the agonists and antagonists do not share common binding sites centrally.

Supported by CTR grant #12130 and NIDA grant #DA07027.

Affiliation: Department of Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298-0613

Diels-Alder Reactions of -New N-Formylmorphinan-6,8-dienes

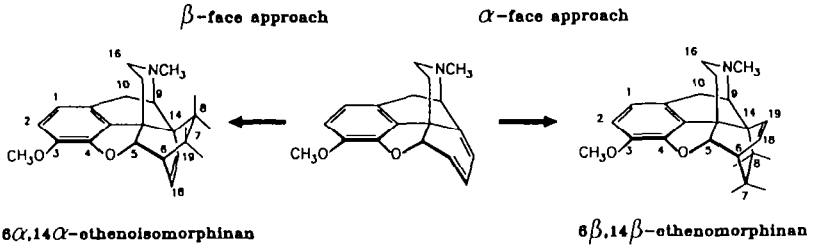
L. Maat, T. S. Lie and J. T. M. Linders

Powerful analgetic 6α,14α-ethenoisomorphinans, such as etorphine, have been prepared by Diels-Alder (DA) reaction of thebaine with mono-substituted ethenes, followed by modification of the substituents. Little is known of the reversal of the reaction pathway. For the preparation of potential antagonists, having a cycloalkylmethyl group at nitrogen, one can envisage an alternative route, namely: N-demethylation of thebaine, *N*-functionalization with the cycloalkylcarbonyl group, the DA reaction and, finally, modification of the relevant substituents. This route has the advantage that base-sensitive dienophiles can be utilized. We have investigated the synthesis and DA reactions of *N*-formylmorphinan-6,8-dienes as a model system.

Thebaine can be converted into *N*-formyl-*N*-northebaine by *N*-demethylation with diethyl azodicarboxylate, followed by formylation with ethyl formate. Application of this procedure to 6-demethoxythebaine gave the *N*-formyl analogue only in moderate yield. The formylation was carried out in the presence of silica-alumina catalysts, such as zeolites, or cracking catalysts. An alternative synthesis of 6-demethoxy-*N*-formyl-*N*-northebaine was devised to start from *N*-formyl-*N*-norcodeine, in analogy with our synthesis of 6-demethoxythebaine from codeine (Ref. 1). Mesylation with methanesulfonyl chloride in pyridine afforded the 6-O-mesyl ester, which was converted into the 8ß-bromo compound by brief treatment with lithium bromide in DMF. Elimination of HBr with the aid of *t*BuOK gave the desired *N*-formylmorphinan-6,8-diene in good yield. When the reaction with LiBr was continued for 5 h, an unexpected rearrangement to (R)-(Z)-7-formyl-8,9-dihydro-2-methoxy-7H-dibenz[d,f]azonine-1-0l was observed. This compound could be obtained also by treatment of 6-demethoxy-*N*-formyl-*N*-northebaine with HBr.

DA reaction of 6-demethoxy-*N*-formyl-*N*-northebaine with methyl vinyl ketone gave the crystalline 7α-acetyl-6α,14α-ethenoisomorphinan in 80% yield. The cycloaddition to the N-methyl analogue also gave this isomer as major product, besides the 8α-adduct and a trace amount of 8ß-acetyl-6ß,14ß-ethenomorphinan, in which the dienophile had approached the diene system from the more

hindered o-face (Scheme). The DA reaction of nitroethene to *N*-formyl-*N*-northebaine occurred in the usual way, giving the 7α-nitro-6α,l4α-ethenomorphinan in 90% yield with no trace of other isomers.



However, the addition of nitroethene to the 6-demethoxy analogue yielded a 5:3:1 mixture of 8α-nitro-6α,14α-ethenoisomorphinan, Bß-nitro-6ß,14ß-ethenomorphinan and 7α-nitro-6α,l4α-ethenoisomorphinan. The different behaviour of this dienophile, when compared to methyl vinyl ketone, can be explained by geometrical and steric factors. Furthermore, it is known that the nitro group is more strongly regio-directing than the acetyl group. An important factor which has to be taken into account as well is the tendency of these 6-demethoxy systems to give 8-substituted adducts. The two types of DA adducts are easily distinguished by means of their ^{1}H NMR spectra. For the 6α,14α-ethenoisomorphinans, the signals for the vinylic protons are typically found between δ 5.3 (H-19) and 6 5.9 (H-18), whereas these protons are found downfield from 6 6.0 in the spectra of the isomeric 6ß,14ß-ethenomorphinans. In the N-methyl derivatives of the latter series, the nitrogen atom has a notable effect on H-19, the signal of which is now found downfield from that of H-18. In the corresponding *N*-formyl compounds, the signals for these protons are found in the usual order. The anisotropic properties of the nitrogen atom are largely responsible for these and other changes, e.g. the influence on 8ß-H (Ref. 2).

The new *N*-formylmorphinan-6,8-dienes give entry to differently substituted 6α,14α-ethenoisomorphinans and the new class of 6ß,14ß-ethenomorphinans (Scheme), which may contribute to a better insight into the conformational and spatial requirements of the lipophilic substituent.

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Department of Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands.

Kappa Antagonist Effects of Buprenorphine in the Rat Drug-Discrimination Procedure

S. Stevens Negus, Mitchell J. Picker and Linda A. Dykstra

Buprenorphine is a partial mu agonist with clinical utility as an analgesic with low abuse potential and, possibly, as an alternative to methadone as a maintenance drug in the treatment of opioid dependence. More recently, evidence has begun to accumulate that buprenorphine binds to kappa opioid receptors as well as to mu receptors. Most of the evidence regarding buprenorphine's activity at kappa receptors suggests that it has little if any intrinsic efficacy at these receptors, and that it therefore acts as a kappa antagonist. The present study was designed to expand on this base of evidence by evaluating buprenorphine's ability to antagonize the discriminative stimulus effects of the selective kappa agonist U50,488 in a two-lever, drug discrimination procedure.

Previous studies have indicated that "perceptual masking" may confound the interpretation of antagonism studies employing the drug discrimination procedure. Perceptual masking is a term borrowed from sensory psychophysics that describes the phenomenon in which the presentation of one stimulus prevents the perception of a second, concurrently presented stimulus. In view of buprenorphine's known mu-agonist effects, it was important to determine in the present study whether any antagonism of U50,488 by buprenorphine could be explained by perceptual masking. Thus, in the present study, buprenorphine could reduce the discriminative stimulus effects of U50,488 by either 1)blocking kappa opioid receptors (antagonism), or 2)producing mu-receptor mediated discriminative stimulus effects that compete with and "mask" the discriminative stimulus effects of U50,488 (perceptual masking). In an effort to differentiate between these two possibilities, the selective mu agonists fentanyl and morphine were also evaluated for their ability to reduce the discriminative stimulus effects of U50,488.

Since morphine, and especially fentanyl, bind with little or no affinity to kappa opioid receptors, any reduction in U50,488's discriminative stimulus effects produced by these drugs should serve as an index of the vulnerability of the U50,488 stimulus to perceptual masking by buprenorphine's mu agonist effects. Finally, the non-opioid barbiturate agonist pentobarbital was also evaluated for its ability to reduce U50,488's discriminative stimulus effects in order to determine if non-opioids could also mask the U50,488 stimulus.

Rats were trained to discriminate 5.6 mg/kg U50,488 from water. During substitution tests, both U50,488 (0.3-17.0 mg/kg) and the kappa agonist bremazocine (0.01-0.3 mg/kg) produced dose-dependent increases in drug-appropriate responding. In contrast, the mu opioid agonists buprenorphine (0.01-0.3 mg/kg), fentanyl (0-03-0.3 mg/kg) and morphine (1.0-10.0 mg/kg) and the non-opioid barbiturate agonist pentobarbital (1.0-10.0 mg/kg) produced primarily water-appropriate responding when given alone. When given in combination with the U50,488, buprenorphine produced a dose-dependent and complete antagonism of the discriminative stimulus effects of the training dose of U50,488. Furthermore, a dose of 0.1 mg/kg buprenorphine shifted the U50,488 generalization curve approximately one half log unit to the right. These results are consistent with the hypothesis that buprenorphine has antagonist activity at kappa opioid receptors. However, fentanyl, morphine and pentobarbital also antagonized the discriminative stimulus effects of U50,488, although to lesser extents than buprenorphine. This suggests that at least part of buprenorphine's kappa antagonist effects in this procedure may result from perceptual masking rather than from blockade of kappa opioid receptors.

S. Stevens Negus, Mitchell J. Picker and Linda A. Dykstra; Dept. of Psychology: CB#3270; University of North Carolina, Chapel Hill, NC; 27514

Fluorescent Probes for Peripheral-Type Benzodiazepine Receptor Visualization and Localization

A. H. Newman, P. Arora, B. McRae, R. T. McCabe and P. Skolnick

Saturable, high affinity sites for benzodiazepines have been identified in the peripheral tissues, transformed cells of neuronal origin, and in some areas of the central nervous system, with physical and pharmacological characteristics that are distinct from classical benzodiazepine receptors. These peripheral-type benzodiazepine receptors (PBR) occur in many peripheral tissues but have discrete localizations as indicated by autoradiography. The prototype ligands for the PBR, Ro 5-4864 and PK 11195 have been shown to bind with high affinity to lymphocytes. The objectives of this study were to prepare a series of PBR selective fluorescent ligands, to determine whether they bind to both rat kidney membranes and mouse spleen cells, and to use fluorescent microscopic techniques for visualization.

A series of fluorescent PBR ligands were prepared based on Ro 5-4864 and PK 11195. The fluorescent moieties nitrobenzoxadiazole (NBD) or dansyl chloride were used to derivatize the parent compound with varying chain lengths (2-6 methylene groups) between the fluorescent moiety and the pharmacophore, to achieve maximal binding to PBR. AHN 590, An NBD-derivative of PK 11195, exhibited moderate affinity (IC_{50} 90 nM) to PBR as evidenced by competition studies with [^{3}H]Ro 5-4864 binding in rat kidney. Preliminary fluorescent binding studies in mouse spleen cells show that these ligands bind and fluoresce. Inhibition of this binding with PK 11195 will determine if this binding is specific to PBR. Further studies in lymphocytes using

these fluorescent PBR ligands may shed light on a possible role for PBR in the modulation of the immune system. In addition these ligands may represent an alternative to radioligands to characterize PBR as well as to study binding kinetics.

AUTHORS

Amy Hauck Newman, Department of Applied Biochemistry, Walter Reed Army Institute of Research, Washington D. C.

Prince Arora, Brad McRae, R. Tyler McCabe, Phil Skolnick, Laboratory of Neurosciences, NIDDK, NIH, Bethesda, MD

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The Effect of Methadone In Vitro on Natural Killer (NK) Activity

Miriam Ochshorn, Gershon Bodner, David M. Novick and Mary Jeanne Kreek

Numerous immunologic abnormalities have been reported to occur in heroin addicts and former addicts in treatment. In earlier studies from this laboratory, we have shown that very high concentrations of both the active (-) and inactive (+) enantiomers of the opioid antagonist naloxone significantly reduce natural killer (NK) cell activity in vitro, but that lower concentrations, such as those used in human therapeutics or to block opioid receptors in clinical research have no effect on NK activity (1). In clinical studies of unselected methadone maintained patients in treatment from one month to ten years, without or with ongoing alcohol, cocaine or other polydrug abuse, we have found normal NK activity in over 50% of patients, whereas studies by our group and others have shown reduced NK activity in essentially all active heroin addicts (2). In a recent, controlled clinical study of very long term methadone maintained patients, each in continuous methadone treatment for over 11 years, none with any ongoing alcohol or drug abuse, and all anti-HIV negative, it was found that all of the long-term methadone maintained patients had normal NK activity. In contrast, the active heroin addicts, who were also anti-HIV negative, had low NK activity (3). We have asked the question of whether methadone, a synthetic long-acting opioid agonist, may affect NK cell cytotoxicity activity.

Eight normal subjects (4 males, 4 females; mean age 28.5y, age range 22-41y) and eight methadone maintained subjects (length of treatment from 18 days to 20y); (7 males, 1 female; mean age 34.3y, age range 18-51y; mean methadone dose 52.5mg, methadone dosage range 30-85mg) were studied. All bloods were drawn between 8:30AM-9:30AM. Methadone maintained patients were studied prior to daily dose of methadone. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Paque density gradient centrifugation. PBHC were incubated for 1 hour without or with a wide concentration range ($1x10^{-13}$ to $1x10^{-3}$M) of (-)methadone, the active enantiomer of methadone, or with (+)methadone, the inactive enantiomer, prior to and during NK activity assay.

The results showed no effect of methadone in concentrations of $1x10^{-13}$ to $1x10^{-5}$M, while very high concentrations ($1x10^{-4}$ to

$1x10^{-3}$M) of either enantiomer of methadone resulted in a significant reduction of NK activity. We conclude that:
1. High concentrations of (-)methadone and also (+)methadone lower NK cell activity, with significant reduction in NK activity observed at methadone concentrations of $1x10^{-4}$M and above.
2. No significant decrease or increase in NK activity results from addition of other contrentrations of (-)methadone or (+)methadone ($1x10^{-5}$ to $1x10^{-13}$M) .
3. Both (+) and (-)methadone at high concentrations have similar effects on NK activity; therefore this effect does not seem to be mediated by any classically defined opioid receptor.

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Affiliation:

The Rockefeller University, New York.

Effects of Mixed-Action Opioids on Food-Maintained Behavior of Morphine-Pretreated and Morphine-Tolerant Rats

Alison Oliveto, Mitchell Picker and Linda A. Dykstra

The effects of various opioid compounds were examined i-n rats responding under a fixed-ratio 30 schedule of food presentation. Dose-effect curves were determined in untreated rats and in rats pretreated with 5.6 mg/kg of morphine 5-6 hr prior to the session. In addition, dose-effect curves were determined in rats maintained on 30.0 mg/kg of morphine daily. In morphine-pretreated rats, the dose-effect curves for the mu-opioid agonists morphine and 1-methadone were unaltered from those determined in untreated rats, whereas those for the opioid antagonists naloxone, naltrexone, and diprenorphine shifted at least 0.5, 0.75, and 2 log units to the left, respectively. The does-effect curves for the mixed-action opioids nalorphine, nalbuphine, butorphanol, pentazocine and bremazocine in morphine-pretreated rats were not different from those determined in untreated rats. In rats chronically maintained on morphine, the dose-effect curve for morphine shifted almost 0.5 log unit to the right, whereas the dose-effect curve for the kappa-opioid agonist U50,488 was unaltered from that determined in untreated rats. The dose-effect curves for naloxone and the mixed-action opioid nalorphine were shifted approximately 2.5 and 1.5 log units to the left, respectively; whereas that for the mixed-action opioid butorphanol was flattened. The dose-effect curves for the mixed-action opioids pentazocine, nalbuphine and bremazocine in morphine-maintained rats were not different from those determined in untreated rats. These results indicate that a chronic morphine administration procedure can be employed to differentiate mixed-action opioids from opioid agonists in rats. (Supported by USPHS Research Grants DA02749 and DA05314)

AFFILIATIONS

Alison Oliveto, Human Behavioral Pharmacology Laboratory, Department of Psychiatry, University of Vermont, Burlington, VT 05401, Mitchell Picker and Linda Dykstra, Psychology Department, University of North Carolina, Chapel Hill, NC 27599

Rat Pups Exposed to Morphine In Utero

J. E. Olley, G. K. L. Tiong, N. M. von Jenner and J. Scheer

Morphine was administered in the drinking water (Badawy et al., 1982) to female Long Evans rats. Daily fluid consumption and growth were measured. Physical dependence, assayed by naloxone-precipitated withdrawal (Wei et al., 1973), was developed by rats taking 0.2 or 0.4 mg/ml morphine (n=4/group). Further groups of rats (n=16) reached maintenance levels of 0.2, 0.4 and 0.8 mg/ml morphine 1 week prior to mating and continued drinking morphine-water throughout pregnancy. Pups were fostered to lactating untreated females within 24 h of birth.

Morphine-exposed pups were cyanotic, hypothermic, had poorer survival rate and weight-gain than controls. Behavioral teratology techniques (Buelke-Sam et al., 1985) applied in the first 21 days indicated that rat pups were small for their age, showed delayed reflex development (righting and negative geotaxis) but earlier occurrence of physical events (eye opening and incisor eruption). Tail-flick response to 50 °C and brain opioids appeared normal on day 24.

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AFFILIATION

Department of Pharmacology, Monash University, Clayton Victoria 3168, Australia

The Effects of Phencyclidine on the Pituitary-Adrenal Axis are Centrally Mediated in the Rat

Robert N. Pechnick, Bonnie M. Chun, Robert George and Russell E. Poland

A common characteristic of many abused drugs is that they markedly disrupt neuroendocrine function and produce changes in plasma and tissue levels of hormones. Phencyclidine (PCP) stimulates the pituitary-adrenal axis in the rat, inducing the release of ACTH and corticosterone (Pechnick et al., 1989). However, the site or sites at which PCP produces these effects is not known. The present study examined the effects of PCP on the different units of the pituitary-adrenal axis in the rat. In addition, PCP was injected directly into the lateral cerebral ventricles in order to determine if the effects of PCP on the pituitary-adrenal axis are centrally-mediated.

PCP (1.0 - 20.0 mg/kg) produced dose-dependent increases in plasma levels of both ACTH and corticosterone when measured 60 min after s.c. administration. However, PCP did not stimulate the release of corticosterone in hypophysectomized rats. To assess the direct effects of PCP on the release of corticosterone from the adrenal, adrenal cells were isolated using the method of Sayers et al. (1971). PCP (10^{-9} - 10^{-3} M) did not affect the basal release of corticosterone from dispersed adrenal cells *in vitro;* however, PCP inhibited ACTH-induced corticosterone release from the adrenal cells. These results demonstrate that PCP has no direct stimulatory effect on the adrenal, and the PCP-induced corticosterone release that occurs *in vivo* must be secondary to the release of ACTH from the pituitary.

In order to determine whether PCP directly stimulates the release of ACTH from the pituitaries, whole rat pituitaries were removed and placed in a superfusion apparatus. PCP (10^{-5} M) had no effect on either the basal or the CRF-induced release of ACTH from superfused pituitaries *in vitro,* indicating that PCP does not act directly at the level of the pituitary. In order to ascertain whether the the effects of PCP on the pituitary-adrenal axis are centrally-mediated, PCP was injected directly into the lateral cerebral ventricles via chronically implanted cannulae. PCP (10.0 - 200.0 µg) produced dose-dependent increases in plasma

ACTH levels when measured 30 min drug injection.

The results of these studies demonstrate that PCP has no directly stimulatory effect on either the pituitary or the adrenal. Thus, PCP stimulates the pituitary-adrenal axis *in vivo* by acting at a site within the central nervous system, leading to the subsequent release of ACTH from the pituitary.

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AFFILIATION

Department of Pharmacology and the Brain Research Institute, U.C.L.A. School of Medicine, Los Angeles, CA. 90024-1735, and Division of Biological Psychiatry, Harbor-U.C.L.A. Medical Center, Torrance, CA 90502 (R.E.P.).
Supported by NIDA grants DA-04113 and DA-05448.

Effects of Naloxone and Mr 2266 on Thermonociceptive Reactions in Diabetic Mice

K. Ramabadran, M. Bansinath, H. Turndorf and M. M. Puig

Response to opiates is affected by glucose homeostasis. The hypoglycemic hormone insulin, has been shown to induce hyperalgesia (Goodman and Soliman, 1989). The decreased opiate sensitivity in diabetes is considered to be primarily due to hyperglycemia (Brase and Dewey 1988; Lux et al 1988). However, some results do not support this hypothesis (Ramabadran *et al* 1989a). The hyperglycemic conditions do not alter the analgesic potencies of opiates like methadone and propoxyphene (Brase and Dewey 1988). Additionally, opiate induced inhibition of gut transit (Bansinath *et al* 1988, Ramabadran *et al* 1989b), thermic responses (Bansinath *et al* 1989a) and mydriasis (Bansinath *et al* 198%) are not uniformly affected in hyperglycemic conditions. In this study, in order to provide new insights into the specific receptor site(s) at which hyperglycemia affects the opiate sensitivity, we assessed the hyperalgesic response of naloxone and Mr 2266.

MATERIALS AND METHODS

Mate Swiss Webster mice (25-30 g) were used. Chronic hyperglycemia (diabetes) was induced by streptozocin (Bansinath *et al* 1989a). Naloxone hydrochloride (3 mg/kg) and Mr 2266 (3 mg/kg) (gift from Dr. H. Merz, Boehringer Ingelheim, F.R.G.) were injected (s.c.) in a volume of 10 ml/kg. A minimum of 10 animals were used per group. The details of the hot plate (Ramabadran *et al* 1989a) and tail immersion test (Ramabadran *et al* 1989c) procedures used were essentially similar to those published earlier. In the hot plate test, the reaction latency in drug treated mouse was calculated as percentage of mean reaction latency of the concomitant control group. In the tail immersion test, post-drug latencies were calculated as percentage of the respective animal's control latency.

RESULTS

In both tests, in normoglycemic mice, naloxone and Mr 2266 induced significant hyperalgesic response (Table 1). In streptozocin treated mice, the hyperalgesic response of naloxone was attenuated, while that of Mr 2266 was not affected.

TABLE 1

	Hot plate[a]		Immersion test[a]	
Drug	Normo	STZ	Normo	STZ
Naloxone	54±4*	100±12	62±6*	106±11
Mr 2266	59±6*	61±8*	56±6*	64±10*

[a] Mean (± SEM) response latency expressed as % of controls; * $P < 0.05$ with respect to their corresponding controls; Normo = Normoglycemic mice; STZ = Streptaocin treated min.

DISCUSSION

Results of the present study indicate that *k* receptor-mediated thermonociceptive mechanism(s) are tonically active at spinal and supraspinal levels. Furthermore, chronic hyperglycemia fails to affect the hyperalgesic response of *k* opioid antagonist Mr 2266 but attenuates the hyperalgesic response of naloxone. Chronic hyperglycemia has been shown to attenuate the inhibition of the gastrointestinal motility induced by the *k* opiate agonist, U-50488H (Ramabadran *et al* 1989b). Taken together, these results suggest that the hyperglycemia induced-changes in the pharmacodynamics of *k* and other opiate receptor selective agonists and antagonists may be site specific. These results support the hypothesis that elevated glucose levels may not be the primary mechanism for the altered opiate response in the experimental models of diabetes (Bansinath *et al* 1988, 1989a,b; Ramabadran *et al* 1989a,b).

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AUTHORS

K. Ramabadran, Ph.D., M. Bansinath, Ph.D., H. Turndorf, M.D., and MM. Puig, M.D., Ph.D. Department of Anesthesiology, NYU Medical Center, School of Medicine, 550 First Avenue, New York, NY 10016

Characterization,of NMDA-Coupled and Dopoamine Reuptake Carrier Coupled [^{3}H]-TCP Binding Sites in Guinea Pig Brain

A. A. Reid, J. A. Monn, A. E. Jacobson, K. C. Rice and R. B. Rothman

INTRODUCTION

Phencyclidine (PCP) has been shown to produce psychotomimetic effects, as well as therapeutic effects, including anticonvulsant and neuroprotective actions. PCP has also been reported to possess a variety of biochemical actions including noncompetitive inhibition of the effects mediated by the glutamate excitatory amino acid receptor specifically activated by N-methyl-D-aspartate (NMDA) and inhibition of dopamine (DA) reuptake. Most studies have reported the presence of a single high affinity PCP binding site coupled to the NMDA receptor complex. However, we recently demonstrated that the PCP analog, [^{3}H]-TCP, labelled two high affinity binding sites in guinea pig brain distinguished by (+)MK801. In the present study we conducted kinetic experiments to evaluate the modulation of the MK801-sensitive (site 1) and MK801-insensitive (site 2) binding sites by NMDA receptor agonists and antagonists and tested the hypothesis that site 2 is associated with the DA reuptake carrier in guinea pig brain.

CHARACTERIZATION OF [^{3}H]TCP BINDING SITES

PCP binding sites were characterized in whole guinea pig brain including cerebellum with [^{3}H]TCP utilizing binding surface analyses. The data best fit a two site binding model. The best-fit parameter estimates yielded Bmax values of 631±10 (site 1) and 829±24 (site 2) fmol/mg protein and Kd values of 14.1±0.2 nM (TCP, site 1), 46.5±1.3 nM (TCP, site 2), 3.22±0.06 nM [(+)MK801, site 1], 5208±342 nM [(+)MK801, site 2]. about 1 mM [BTCP (TCP analog), site 1] and 1083 ±167 nM (BTCP. site 2). These analyses indicate that TCP is essentially nonselective between the two PCP sites, while (+)MK801 is 1600 fold selective for PCP site 1 and BTCP is about 1000 fold selective for PCP site 2.

KINETIC EXPERIMENTS

Kinetic experiments were conducted to examine the ability of NMDA

receptor agonists and antagonists to facilitate dissociation of [^{3}H]TCP bound to the PCP sites. The dissociation of [^{3}H]TCP from site 1 and site 2 proceeded very slowly. Mg (100 µM) markedly enhanced the dissociation of [^{3}H]TCP from site 1. The combination of Mg and AP7 (100 µM each) resulted in a reversal of Mg's effects at site 1, while the combination of Mg/AP7/Glutamate (100 µM each) produced the same increased rate of dissociation of [^{3}H]TCP from site 1 as observed with Mg alone. These agents were essentially ineffective at PCP site 2.

DA REUPTAKE BLOCKER STUDIES

Evaluation of the association of PCP site 2 with the DA reuptake carrier involved BTCP and GBR12909, two potent inhibitors of DA reuptake.Guinea pig caudate membranes were preincubated with varying concentrations of the two drugs and then washed twice by centrifugation prior to analysis of [^{3}H]TCP binding sites. Neither drug had any significant effect on PCP site 1, except 10,000 nM BTCP, which inhibited [^{3}H]TCP binding to this site by 20%. BTCP produced a dose-dependent wash-resistant inhibition of [^{3}H]TCP binding to PCP site 2 at concentrations as low as 100 nM (20% inhibition), while GBR12909 required higher concentrations (1000 nM, 46% inhibition).

CONCLUSIONS

The kinetic experiments demonstrated that glutamate, a NMDA receptor agonist, Mg, a noncompetitive NMDA receptor antagonist and AP7, a competitive NMDA receptor antagonist modulated [^{3}H]TCP binding to PCP site 1 but not PCP site 2, which suggests that PCP site 1 is coupled to the NMDA receptor complex. Preincubation of guinea pig striatal membranes with varying concentrations of the high affinity DA reuptake inhibitors, GBR12909 and BTCP, caused a dose-dependent, wash-resistant inhibition of [^{3}H]TCP binding to PCP site 2 but not PCP site 1. This tight binding phenomena suggests that PCP site 2 is associated with the DA reuptake complex. These data raise the possibility that PCP's behavioral effects might be separately mediated via the two binding sites: the psychotomimetic effects could be mediated via the DA uptake carrier site, since an increase in the release of DA has been associated with psychotic behavior and the therapeutic properties could be linked to PCP's noncompetitive inhibition of NMDA-receptor mediated effects.

REFERENCES available upon request from author.

AFFILIATIONS

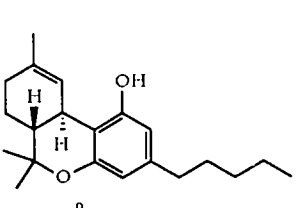
Laboratory of Medicinal Chemistry, NIDDK and Unit on Receptor Studies, LCS, NIMH, NIH, Bethesda, MD 20892.

Progress Toward the Synthesis of Potential Affinity Ligands for the Analgesic Cannabinoid Receptor Based on CP-55,244

Scott Richardson, Miles Herkenham, Seid Mirsadeghi, M. Ross Johnson, Larry S. Melvin and Kenner C. Rice

The major pharmacologically active constituent of the marijuana plant *(Cannabis sativa)* is Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**) and for many centuries, marijuana preparations have been used as medicinal agents. Ancient physicians noted many clinically useful effects of marijuana, and among these, prescribed marijuana as an antiemetic and analgesic. Currently, synthetic Δ^9-THC is used as an antiemetic for patients undergoing cancer chemotherapy.

In 1976, seminal studies by Wilson et *al* revealed that 9-nor-9-ß-hydroxyhexahydrocannabinol (HHC, **2**) was equipotent with morphine as an analgesic in mice and later investigations indicated a nonopioid mechanism of action. Recent biological studies on CP-55,244 (**3**), prepared at Pfizer, have revealed this drug to be an extremely potent ligand for the newly discovered analgesic cannabinoid receptor. These results have prompted us to use CP-55,244 as a template for the design of chemical probes to further study the structure and function of this system. We intend to use the ketone **4** as a common intermediate in the syntheses of analogs of CP-55,244 with electrophilic functionality in specific regions of the parent structure. A summary of our progress in these synthetic studies will be described.



Δ^9-THC

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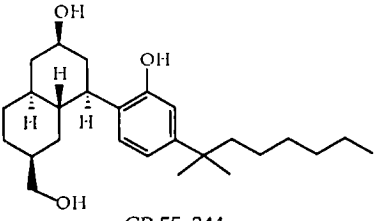

CP-55, 244

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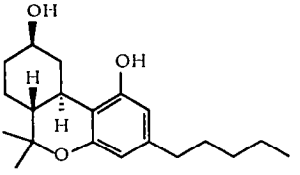
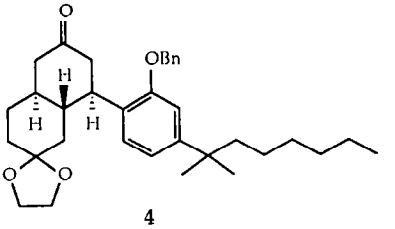


HHC



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AFFILIATIONS

Laboratory of Medicinal Chemistry, National Institute of Digestive and Diabetes and Kidney Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892
National Institute of Mental Health, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892
Pfizer Central Research, Eastern Point Road, Groton, CT 06340
Glaxo, Inc., Five Moore Road, Research Triangle Park, NC 27709

Excitatory Amino Acid Antagonists as Well as GABA Agonists Cause Barbiturate-Like Anesthesia in Rats

J. A Richter and S. L. Gatto

We are interested in determining the mechanism of barbiturate anesthesia. Since barbiturates are known both to mimic and enhance GABA actions and to inhibit glutamate actions, synapses using either of these transmitter systems could be the neurochemical site for this barbiturate effect. In order to evaluate these potential mechanisms, we have tested other drugs known to be GABA agonists or excitatory amino acid (EAA) antagonists on rats in vivo to determine if they can cause anesthesia and/or other effects also induced by barbiturates.

Male Wistar rats were implanted unilaterally with intraventricular (ivt) guide cannulae. After recovery, the freely moving animals were injected with a dose of test drug in 10 ul vehicle over 15 sec. CNS depression was evaluated on a 7 point scale (0=normal to 7=coma, modified after Freer and Gallagher). Rectal temperature and respiratory rate were also measured. The rats were randomly assigned to treatment and the observer was blind to the test drug/dose. Each animal was used only once. After testing the injector placement was confirmed by dye injection; data are reported only for those rats in which the injection was into the lateral ventricle.

Phenobarbital (PheB) was tested at BOO, 1200 and 1600 ug ivt. As previously described by Mycek and Brezenoff (1976) it induced a dose-related, brief period of CNS depression up to a score of at least 6 (loss of righting reflex and only a flinch to a tail pinch) and a brief decrease in rectal temperature. The GABA agonist muscimol (tested at 0.3, 1 and 3 ug) caused long-lasting CNS depression to a score of 6 and very large, long-lasting decreases in temperature at the two highest doses. The N-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphono-valieric acid (APV) was tested at 30, 100, 300 and 1000 ug. The three higher doses caused CNS depression to scores of 6-7; after recovery to a score of 1 (ataxia) the ataxia was notably long lasting with this agent. The temperature decrease caused by APV was somewhat smaller than that caused by PheB at the same CNS depression score. Two non-NMDA antagonists were examined: 6-D-glutamyl-

aminomethylsulfonate (GAMS) at 240 and 480 ug and 6,7-dinitroquinoxaline-2,3-dione (DNQX) at 25 ug. These agents also depressed the CNS to a score of at least 6; at these doses they induced relatively larger decreases in temperature than APV.

Respiratory rate (measured by counting chest excursions) was decreased below the normal rate of about 100/min at the highest doses of PheB and muscimol. APV did not depress respiratory rate at the doses used. DNQX also did not depress respiratory rate at the dose tested and the effect of GAMS was modest at the higher dose used. All of the drugs tested caused muscle relaxation at the highest doses in that the animals became "limp". No catalepsy was observed with any of these agents given ivt.

These results demonstrate that the EAA antagonists tested have a spectrum of effects similar to the GABA agonist muscimol and to the barbiturate phenobarbital. APV at anesthetic doses seemed to have relatively lesser effects on temperature and respiration than the other agents, however it also caused a longer residual ataxia. The finding that the EAA antagonists do cause CNS depression resembling that induced by phenobarbital indicates that barbiturate anesthesia might be induced by antagonizing EAA actions just as well as by mimicking or enhancing GABAergic inhibition. We have some in vivo evidence for the GABAergic mechanism in that anesthesia induced by systemic PheB can be antagonized by local administration of bicuculline or picrotoxin in the posterior hypothalamus (Richter and Glick, 1987). However analogous tests of the EAA mechanism are still required. (Supported by PHS Grant DA 00796).

AFFILIATION

Departments of Pharmacology and Psychiatry (Institute of Psychiatric Research), Indiana University School of Medicine, Indianapolis, IN 46202-4887.

Discrimination of the Benzodiazepine Receptor Antagonist Ro 15-1788 Using the Conditioned Taste Aversion Procedure

Grace A. Rowan and Irwin Lucki

Rats were trained to discriminate the stimulus properties of the benzodiazepine receptor antagonist flumazenil using a conditioned taste aversion procedure. Fluid-restricted rats were injected with flumazenil (33 mg/kg) or saline and then given access to a 0.25% saccharin solution for 30 minutes. When rats (N=15) received a drug trial, saccharin consumption was followed by an injection of LiCl (1.8 mEq/kg i.p.), while on saline trials saccharin consumption was followed by a second saline injection.

Acquisition of the discriminated taste aversion, as measured by the differential effects on saccharin drinking between drug and saline trials, developed after only five pairings of the flumazenil with the LiCl injections. Unconditioned controls (N=9), that received an identical sequence of drug and saline trials, but never received LiCl, did not show differences in saccharin intake. The discrimination was also verified using two-bottle preference measures between saccharin and water.

Flumazenil demonstrated dose-dependent generalization upon varying the training dose. Two other benzodiatepine antagonists of different classes, CGS-8216 and ZK 93426, also demonstrated complete substitution for the flumazenil stimulus. Partial generalization was exhibited with the inverse agonists FG 7142 and BCCE, while PTZ failed to generalize to the flumazenil stimulus. The benzodiazepine agonists Diazepam and Alprazolam also failed to generalize to the flumazenil stimulus. This research was supported by DA05367 and DA05186.

The Department of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA.

Cation Requirement for GTP Regulation of [^{125}1]b-Endorphin Binding to Mu and Sigma Opioid Receptors

D. E. Selley and J. M. Bidlack

An important focus of drug abuse research is the difference between the agonist properties of exogenous drugs and those of the endogenous transmitters which they mimick. Inhibition of agonist-receptor binding by guanine nucleotides has been used as an indicator of coupling between receptors and guanine nucleotide-binding regulatory proteins. The binding of alkaloids and modified enkephalin agonists to μ and δ opioid receptors is inhibited by μM concentrations of GTP. Since the endogenous opioid peptide, ß-endorphin (ß-EP), activates both μ and δ opioid receptors, we investigated the ability of GTP to inhibit [^{125}I]ß-EP binding to opioid receptors in rat brain membranes. Unlike the binding of the μ- and δ-selective peptides, .[^{3}H][D-Ala^{2}, MePhe^{4}, GLY-ol^{5}]enkephalin and [^{3}H][D-Penicillamine^{2}, D-Penicillamine^{5}]enkephalin respectively, [^{125}I]ß-EP binding was not altered by GTP in the absence of cations. However , in the presence of NaCl, [^{125}I]ß-EP binding was inhibited by GTP in a concentration-dependent manner. This Na effect on GTP regulation of binding was mimicked by Li^{+} and K^{+}, with the order of potency being: $Na^{+} > Li^{+} > K^{+}$, but not by the divalent cations, Mg^{2+}, Ca^{2+}, and Mn^{2+}. Similar experiments were conducted in membrane preparations from two cell lines: NG108-15, which contain only δ opioid receptors, and SK-N-SH, which contain predominantly μ opioid receptors. As in the rat brain membranes, [^{125}I]ß-EP binding to SK-N-SH membranes was not affected by GTP in the absence of cations, but was inhibited by GTP in the presence of monovalent cations, with the order of potency being: $Na^{+} > Li^{+} > K^{+}$. [^{125}I]ß-EP binding to NG108-15 membranes was also not inhibited by GTP in the absence of cations. In this cell line, however, only Na^{+} promoted inhibition of [^{125}I]ß-EP binding by GTP, with Li^{+} and K^{+} having no significant effect. The divalent cations, Mg^{2+}, Ca^{2+}, and Mn^{2+} did not promote inhibition of [^{125}I]ß-EP binding by GTP in either of these cell lines. These results indicate that monovalent cations are required for guanine nucleotide regulation of ß-EP binding, and that the specificity of this requirement differs among tissues containing different relative numbers of μ and δ binding sites. The δ site specifically requires sodium in order to express guanine nucleotide regulation of [^{125}I]ß-EP binding. Guanine nucleotide regulation

of [^{125}I]ß-EP binding to the μ site occurs in the presence of sodium, lithium, or potassium, with sodium being the most potent promotor of this effect.

ACKNOWLEDGEMENTS: Supported by USPHS grants DA05319 and DA03742 from the National Institute on Drug Abuse.

AFFILIATIONS: Department of Pharmacology, The University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

The Effects of Capsaicin Treatment on Self-Administration of Amphetamine Vapor in Rats

L. G. Sharpe, L. L. Weinhold and J. H. Jaffe

Drug inhalation is an increasingly popular way to self-administer psychoactive drugs. Smoked cocaine (crack) use has increased 4 fold between 1985 to 1988 in the USA (DAWN), providing an incentive to develop an animal model for the self-administration of non-volatile drugs.

We recently reported that tats self-administered nebulized sufentanil (10 to 75 ug/ml) in a concentration-dependent manner (Jaffe et al. Psychomacology 1989). However, the less potent psychoactive drugs (i.e. morphine. amphetamine and cocaine) have failed to maintain responding for drugs in vapor form. The present experiments were conducted to determine whether capsaicin pre-treatment, which depletes substance P, facilitates self-administration of amphetamine and cocaine aerosol by rats.

Rats (Sprague-Dawley) were either treated with capsaicin (n = 13) or not treated (n=14) before exposure to amphetamine (5, 10 mg/ml) or cocaine (2.5, 5 mg/ml) in vapor form. Capsaicin doses were 75 and 125 mg/kg given over a 3-day period. Drug vapor self-administration was accomplished under FR schedules where nose-poking behavior resulted in a 2-sec aerosol presentation with subsequent 60 sec black-out periods. Drugs were delivered by a commercial nebulizer through a hose connected to the vapor chamber. Sessions were conducted for 3 hr or overnight (13-15 hr).

Response rates revealed that: (1) In several rats, capsaicin treatment facilitated the self-administration of amphetamine vapor but not of cocaine vapor; (2) Our best strategy for training rats to self-administer cocaine in aerosol form was to allow access to 2.5 mg/ml cocaine vapor during overnight sessions (13 to 15 hr); (3) The reported dose differential between amphetamine and cocaine self-administered intravenously (about 1 to 10), was not observed in rats self-administering amphetamine and cocaine in aerosol form.

Further investigations of capsaicin treatment are required to conclude that capsaicin treatment alters self-administration of amphetamine and cocaine in aerosol form.

Affiliation: NIDA, Addiction Research Center, Baltimore, MD 21224

One-Trial Conditioned Rotation in Rats

Peter B. Silverman

The association of drug effects with specific environmental stimuli is probably importantly involved in drug abuse *concerns* such as relapse. We have found that in addition to the well characterized rotational behavior that follows the acute administration of dopamine agonists in rodents with unilateral nigrostriatal lesions, extraordinarily persistent conditioned rotation can be demonstrated, often after a single drug treatment (and thus a single pairing of drug and environment). In the work described here, the rotational behavior of lesioned rats was tested before, shortly after, and weeks after the administration of direct and indirect dopamine agonists.

Sprague-Dawley rats of either sex were anesthetized with sodium pentobarbital and positioned in a stereotaxic frame. They were lesioned by injection of 6-hydroxydopamine aimed at one substantia nigra. Two to 4 weeks after being lesioned, rats *were* individually placed in a clear plastic hemispherical bowl for a 3 min undrugged observation period during which 360 degree turns in either directions were counted by an observer. They were then injected with one of the compounds of interest, placed in the bowls, and circling was counted and recorded in 3 min bins. These 3 min observation periods were at intervals after injection selected to include the peak drug effect as determined in pilot work. In some cases the sequence of undrugged observation followed by drug administration and observation was repeated on consecutive days or at longer intervals. In other instances drug was given only once. In either case, undrugged observations were made prior to drug treatment and subsequently weeks after the treatment. In the interval between tests, animals were left undisturbed in their home cages. In no case was any individual animal treated with more than one drug. The compounds tested to date include both direct (apomorphine and LSD) and indirect (cocaine and (+)-amphetamine) acting dopamine agonists which acutely induce rotation contralateral and ipsilateral to the lesioned side, respectively.

Administration of 0.05 mg/kg apomorphine typically induced peak contralateral rotation of 30-40 turns/3 min at 15 min after injection. When reintroduced to the rotation bowl weeks or months after a single such apomorphine treatment, rats rotate rapidly contralaterally. This conditioned rotation is often more rapid in the brief undrugged test than the peak rate achieved acutely after drug administration. The basic finding of a conditioned drug effect after a single apomorphine treatment has been replicated a number of times. Conditioned rotation is even more rapid and prolonged after repeated small doses of apomorphine administered daily or at longer intervals. A single treatment with 0.2 mg/kg LSD or two treatments with 0.1 mg/kg LSD resulted in acute rotation of 25-40 turns/3 min. Similar to the result with apormorphine, LSD-treated animals subsequently exhibited conditioned rotation when placed, undrugged, in the rotation bowls.

In tests with (+)-amphetamine, 2 to 5 mg/kg administered on 1 to 3 occasions, ispilateral rotation of 20 to 50 turns/3 min was induced acutely. No ispilaterally directed conditioned rotation was ever observed in these animals. Similarly, 10-20 mg/kg cocaine induced acute rotation of 30-60 turns per min at 10 min after injection, but no conditioned rotation was observed in animals that had been drug treated 1 to 3 times.

Thus at doses which induce comparable acute rates of rotation, direct acting dopamine agonists also resulted in a clear conditioned effect while indirect agonists did not. While it cannot be concluded that much more frequently repeated pairings of indirect agonists with the rotation environment wouldn't result in conditioned rotation, the data presented here show relative ease of conditioning with direct agonists. This may suggest greater potential for classical conditioning via supersensitive than normosensitive structures, an implication of importance in drug abuse issues as well as learning in general.

Supported in part by NIDA grant DA 04423.

Affiliation: Substance Abuse Research
Department of Psychiatry - MSI
University of Texas
Houston, Texas 77030

Neural Connectivity in the Descending Pain Pathway

Laura J. Sim and Shirley A. Joseph

Numerous electrophysiological and neuroanatomical studies have provided evidence that the endogenous opiocortin system provides a substrate mediating nocioception. Electrical stimulation or morphine microinjection into the periaqueductal gray (PAG), dorsal raphe nucleus (DRN) or nucleus raphe magnus (NRM) elicits analgesia (1,2,3). This stimulation produced analgesia (SPA) is naloxone reversible and results in an increase in levels of endogenous opioid peptides. Perikarya containing opiocortin peptides can be demonstrated immunocytochemically in two distinct regions, the arcuate nucleus in the hypothalamus (4) and the nucleus tractus solitarius (NTS) of the medulla (5). From our deafferentation immunocytochemical studies (6) we have shown that the fibers emanating from the arcuate distribute to nuclei in the descending pain pathway whereas the medullary opiocortin neurons which contribute little to these areas. Anterograde tracing studies were performed in order to document the terminal fields for these opiocortin peptides.

A 2.5% solution of phaseolus vulgaris leucoagglutinin (PHA-L) was stereotaxically injected into the rat arcuate nucleus. PHA-L was delivered iontophoretically via a glass micropipette at 5µm alternating positive current over 20 minutes. Five to 15 days later rats were perfused or injected with intraventricular colchicine and perfused 24-48 hours later. Animals were perfused intracardially with saline followed by 4% paraformaldehyde in 0.1M acetate buffer (pH 6.5) then 4% paraformaldehyde in 0.1M borate buffer (pH 9.5). Tissue was processed for PHA-L using immunocytochemistry with nickel enhancement to produce a purple/black granular reaction product. Sections were then stained immunocytochemically for appropriate peptides and transmitters with routine chromagen visualization to yield a brown hqmogenous reaction product.

PHA-L labeled fibers and terminals were identified throughout the brain following arcuate injections. The injection site was visualized as a discrete group of neurons within the arcuate that was completely filled with PHA-L reaction product. Dual staining procedures and alternate section analysis with opiocortin (ACTH) immunocytochemistry verified that the PHA-L was incorporated into arcuate opiocortin containing neurons. The PAG contained dense PHA-L immunoreactive (-ir) fibers and terminals, particularly in the ventrolateral region. PHA-L-ir fibers were also identified in DRN, parabrachial nucleus, locus coeruleus, NRM, nucleus reticularis

paragigantocellularis, dorsal motor nucleus of the vagus and NTS. Dual immunocytochemical staining demonstrated that serotonin immunoreactive neurons in the ventrolateral PAG, DRN and NRM were present in close proximity to opiocortin fiber terminals. PHA-L-ir fibers terminated near catecholaminergic elements in PAG, periventricular gray (PVG) and locus coeruleus. Neurons immunoreactive to substance P, cholecystokinin (CCK), neurotensin, enkephalin and corticotrophin releasing factor (CRF) were identified in opiocortin terminal fields in the PAG. In addition to the projections demonstrated to brainstem nuclei, PHA-L-ir fibers were immunostained in telencephalic and diencephalic nuclei innervated by arcuate opiocortin neurons. In the forebrain, labeled fibers and terminals were identified in the amygdaloid complex, nucleus accumbens. lateral septal nucleus and bed nucleus of the stria terminalis. Terminal fields were also identified in the preoptic area, anterior hypothalamus, lateral hypothalamus, dorsomedial hypothalamus, paraventricular nucleus, supraoptic nucleus, PVG, zona incerta, medial thalamus (paraventricular and central medial nuclei) and habenula.

The importance of the endogenous opioid system in a supraspinal nocioceptive mechanism has been documented. Specific regions mediating this phenomenon include medial thalamus, PAG. NRM and DRN. These studies demonstrate endorphinergic/ACTH terminals in brainstem regions specifically associated with serotonergic and catecholaminergic perikarya. This suggests that a synaptic relationship may potentiate the influence of the endogenous opioid peptide on the descending pain pathway by way of monoaminergic systems.

Neuroendocrine Unit, University of Rochester, Rochester, NY 14642

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Opioid Dependence After Continuous.Intrathecal Infusion of Mu and Delta Opioids in the Rat

Craig W. Stevens and Tony L. Yaksh

The continuous intrathecal (i.t.) infusion model in rats has been used extensively in our lab to study the time course of tolerance to spinal antinociceptive agents; the degree of tolerance induced by agents with varying potency; the effects of chronic naioxone administration on i.t. morphine; and cross-tolerance between morphine and dermorphin, morphine and alpha -agonists, and morphine and DADLE *(Stevens and Yaksh, '86, '87; Stevens, et al. '88 Stevens and Yaksh, '89 a,b,c).* Importantly, this model allows the induction of tolerance and dependence in a dose-dependent or posological fashion *(Yaksh and Stevens,* '86). By emplo ing three log-spaced infusion doses for each agent (termed *toleragen)* that are chosen so as to produce equi-effective peak effects after 1 day of i.t. infusion, we can also compare across toleragens.

In rodents, the degree of opioid dependence can be quantified by the appearance of characteristic withdrawal signs *(Way et al. 1969).* Recent studies have shown a spinal site of opioid withdrawal *(Buccafusco and Marshall, '85; Marshall and Buccafusco, '85).* Systemic studies of the spinal expression of opioid dependence, to date, have not been performed. The present study measures dependence as seen after systemic naloxone in rats made tolerant to mu and delta opioids.

The continuous i.t. infusion of morphine (2, 6, or 20 nmol/h), sufentanil (0.06, 0.2, or 0.6 nmol/h), DAMGO (0.1, 0.3, or 1.0 nmol/h), or DADLE (2, 6, or 20 nmol/h) in awake rats produces a dose-dependent increase in hot plate latency one day after pump implant followed by a gradual return to baseline values by days 3-4; i.e. tolerance. Rats given naloxone (1 mg/kg, i.p.) after 7 days i.t. infusion show a opioid withdrawal syndrome most noted by withdrawal body shakes (WBS). The number of WBS was proportional to the infusion dose and although each opioid was given in equi-effective infusion doses, the agents varied in the degree of dependence noted. Thus, as shown in the table, highly potent agents produce less dependence. This is likely due to the lesser receptor occupancy of potent agents (e.g. DAMGO) to produce its effect as compared to less potent agents (e.g. morphine). The mu and/or delta nature of spinal withdrawal remains inconclusive, although DADLE did produce the highest levels of WBS.

Table 1. Opioid dependence after 7-day continuous infusion of mu or delta opioids. Data are mean (sem) of total WBS for 30 min after naloxone administration. Groups not joined by a common underline differ at P<.05 (ANOVA and Newman-Keuls test).

	Infusion Dose		
Toleragen	Low	Medium	High
Morphine	11.5 (1.8)	12.2 (1.7)	30.2 (5.7)
Sufentanil	6.2 (2.2)	11.5 (1.4)	25.0 (4.5)
DAMGO	1.8 (0.6)	4.0 (0.6)	8.3 (1.2)
DADLE	13.5 (2.0)	21.0 (5.2)	32.7 (8.4)

Dose	Lowest → Highest			
Low	DAMGO	SUF	MOR	DADLE
Medium	DAMGO	SUF	MOR	DADLE
High	DAMGO	SUF	MOR	DADLE

The present results support and extend previous studies implicating a spinal site for opioid withdrawal. Further studies are needed with more highly selective and potent delta agonists.

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AUTHORS:

Craig W. Stevens, Ph.D.
Dept. of Cell Biology and Neuroanatomy
University of Minnesota
Minneapolis, MN 55455

Tony L. Yaksh. Ph.D.
Dept. of Anesthesiology
University of California at San Diego
La Jolla, CA 92093

Synthesis of Exo and Endo Mecamylamine Analogs for Nicotinic Antagonism in the CNS

John A. Suchocki, Thomas J. Martin, Everette L. May and Billy R. Martin

Several attempts to prepare the endo isomer of mecamylamine by way of addition of a methyl nucleophile to the methylamine Schiff base of camphenilone were unsuccessful. The Schiff base intermediate, however, was reduced with $LiAlH_4$ to give, endo-2-desmethyl-mecamylamine in 29% yield. Notably, treatment of the Schiff base of camphenilone with iodomethane followed by methyl lithium gave the rearrangement product N-methyl-isobornylamine in 35% yield, which was independently prepared from camphor.

The tertiary N-methyl derivative of mecamylamine was obtained in low yields (35%) by reacting mecamylamine with iodomethane followed by treatment with base, sodium methoxide. The N-methyl derivatives of exo- and endo-2-amino-norbornane were prepared in moderate yields (-60%) by the reduction of the carbamate formed from 2-aminonorbornane and 4-nitrophenyl chloroformate. Another analog of mecamylamine was prepared from 3-methylenenorbornan-2-one by way of hydrogenation of the exocyclic double bond followed by reduction of the methyl amine Schiff base to give N-methyl-3-methyl-2-norbornamine in a 28% overall yield. This product is indicated to be the 2(exo)-3(endo) isomer based upon steric arguements and chromatographic retention factors. X-ray analysis to confirm the stereochemistry of this compound is presently underway. Similarly, optically pure exo and endo isomers of (+) and (-) N-methyl-fenchylamine have been prepared from (+) and (-) fenchone in an overall yield of about 55% for the

exo isomer. An epimeric ratio of 98:2 for exo to endo isomers was calculated.

A series of stereoisomers possessing structural features of both nicotine and mecamylamine were prepared from nicotinaldehyde and optically pure samples of fenchylamine. Structures and configurations were determined by analogy, spectral data and (in one instance) X-ray crystallography. Supported by NIDA grant #DA07027 and CTR grant #2130.

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia, Richmond, Virginia, 23298-0613

Autoradiographic Localization of ^{3}H-Dextromethorphan Binding Sites Differs from NMDA

F. C. Tortella, R. Davey, M. Pellicano and N. G. Bowery

INTRODUCTION

Dextromethorphan (DM), a widely used non-opioid cough suppressant which binds to high affinity sites in brain[4], also possesses anticonvulsant, neuroprotective and antischemic properties[1] 4 [5]. Since DM appears capable of antagonizing the neurophysiological and neuropathological effects of glutamate[1] 2, it has been suggested that this (+) opiate is also an NMDA antagonist. Using quantitative autoradiography, we have visualized the heterogenous localization of the DM recognition site in rat brain and report a distribution of binding sites for ^{3}H-cM that differs from NMDA.

METHODS

Cryostat sections (10 μm; parasagittal) of Wistar rat brains were placed on subbed glass slides stored at -20°C. Prior to labelling, thawed sections were incubated for 30 min at -20°C in 50 mM Tris-HCL buffer, pH 7.4 DM recognition sites were labelled by incubating the sections for 20 min at 20°C in buffer solutions containing 8 nM ^{3}H-DM (70.8 ci/mmole). The sections were then inserted rinsed in fresh solution at 4°C for 2 x 30 sec and dried prior to apposition with LKB Ultrofilm. Exposure time was 4 weeks and the autoradiograms were quantified using a Quantmet 970 image analyzer (Cambridge Inst., PLc). Nonspecific binding was determined by incubation with 10 mM unlabelled DM.

RESULTS AND DISCUSSION

In concurrence with the membrane binding data obtained previausly[4], ^{3}H-DM binding was fully displaced by 10 mM DM or 1 mM chlorpromazine, but not by 10 nM codeine, NMDA, glutamate, APV or MK801 (not show). The highest density of ^{3}H-DM sites was measured in the cerebellar molecular layer (10.9±2.1 nCi/mg tissue) with a much lower level measured in the granular cell layer. The density rank order determined from 4 sections from each of 4 rat brains was the cerebellar molecular layer > thalmus > caudate putamen > cerebral cortex > hippocampus CA1 > pons > cerebellar granular layer. This contrasts with the localization of NMDA sites, as labelled

with ^{3}H-glutamate or ^{3}H-MK801, where the rank order is hippocampus CA1 > cerebral cortex > caudate putamen > thalamus > cerebellar granular cell layer > pons > cerebellar molecular cell layer. In contrast, some similarity with (+)SKF10047 was noted. comparative data are summarizd in Table 1.

Table 1. Regional densities of DM and NMDA binding sites in rat brain.

Values are expressed as a percent of the highest density for each ligand in the regions listed.

Region	^{3}H-DM (I)	^{3}H-Glutamate (II)	(III)	^{3}H-MK801 (IV)	^{3}H-(+)SKF (V)
Cerebellum mol. layer	100	6	<10	21	73
Thalamus	89±5	16-39	20	48	64
Caudate Putamen	79±9	51	21	29	50
Cerebral Cortex	74±7	39-78	22-74	27-54	67
Hippocampus CA1	70±7	100	100	100	100
Pons Medulla	61±5	7	3	7	42
Cerebellum gran. layer	48.6	24	19	21	73

(I)-Present study; (II)-derived from Monaghan & Cotman, 1985, J. Neurosci. 5: 2909; (III)-derived from Maragos et al., 1988, J. Neurosci. 8:493; (IV)-derived from Bowery et al., 1988, Br. J. Pharmacol. 93:944; (V)-derived from Sircar et al., 1986, JPET 237:681.

These result confirm the presence of specific DM binding sites in rat brain[4] and demonstrate that they are heterogenously distributed. However, these data also indicate that the distribution of DM sites bears no resemblance to those of NMDA. Consequently, it would appear unlikely that DM acts directly on the NMDA receptor complex as an NMDA antagonist. On the other hand, the similarity between the regional localization of DM and the sigma ligand (+)SKF10047 (which competes with high affinity for the DM site[3]) supports the suggestion that these ligands bind to similar high affinity sites in brain.

ACKNOWLEDGEMENTS: We wish to thank ICI Pharmaceuticals, Maaclesfield, England for their generous gifts of ^{3}H-dextromethorphan.

Neuropharmacology Branch, Department of Medical Neurosciences Walter Reed Army Institute of Research, Washigton, DC 20307 and *Department of pharmacology, School of Pharmacy, University of London London ECIN 1AX, England.

REFERENCES: Furnished upon request.

Changes in Prodynorphin Peptide Content Following Treatment with Morphine or Amphetamine: Possible Role in Mechanisms of Action of Drug of Abuse

Keith A. Trujillo and Huda Akil

The discovery of opiate receptors and endogenous opioid peptides led to early speculation that these systems might play an important role in tolerance, dependence and addiction. A number of early studies were therefore aimed at determining how opiate receptors and opioid peptides were altered in the brain in response to treatment with drugs of abuse, particularly the opiate alkaloids. These studies. were disappointing, however, in that no consistent changes were seen in either opiate receptors, enkephalins, or endorphins in response to chronic treatment with these drugs.

The prodynorphin family is the most recently discovered of the endogenous opioid peptide systems. The prodynorphin (Prodyn) precursor codes for three opioid peptide domains: dynorphin A (Dyn A), dynorphin B (Dyn B) and Neo Endorphin (Neo Endo), and at least one non-opioid peptide domain. The opioid domains in the Prodyn precursor can yield a number of peptides, including Dyn A 1-17 and Dyn A 1-8 from the Dyn A domain, leumorphin and Dyn B 1-13 from the Dyn B domain, and a-Neo Endo and ß-Neo Endo from the Neo Endo domain. Due to the relatively recent discovery of the Prodyn family, less is known about its regulation in brain. In order to determine the possible role of Prodyn systems in the mechanisms of action of drugs of abuse we have examined the response of Prodyn systems in the brain to treatment with either morphine or amphetamine. We focused our experiments on the striatonigral Prodyn system, examining drug-induced alterations in peptides in both the dorsal, or "sensorimotor" striatum (caudate-putamen), and the ventral, or "limbic" striatum (nucleus accumbens-olfactory tubercle).

METHODS

Adult male Sprague-Dawley rats were used in all experiments. For chronic morphine administration, rats were implanted with 75 mg morphine pellets or placebo pellets (1 pellet on day one and 3 pellets on day four; pellets obtained from NIDA) under light ether anesthesia, and sacrificed on day seven. For amphetamine administration, rats received a single subcutaneous injection of d-amphetamine sulfate or saline vehicle each day for 7 days, with sacrifice 24 hours after the final injection. Brains were rapidly dissected, the tissues extracted in methanol/HCl, and the extracts examined with radioimmunoassays highly selective for peptides from all three opioid domains in the Prodyn precursor: Dyn A 1-8, Dyn A 1-17, Dyn B 1-13, and a-Neo Endo.

RESULTS AND DISCUSSION

Chronic treatment with morphine increased Prodyn peptides from all three opioid domains, in both the dorsal striatum and the ventral striatum. Increases in the dorsal striatum averaged 60% above control animals, while increases in the ventral striatum averaged only 20% above control animals. Similarly, repeated amphetamine administration increased all Prodyn peptides assayed, in both the dorsal and ventral striatum. As with chronic morphine treatment, increases were greater in the dorsal striatum than in the ventral striatum, ranging from 57% above control to 92% above control in the former and averaging 30% above control in the latter. Although previous work suggests that the effects of repeated amphetamine are mediated by activation of mesostriatal dopamine systems, leading to increased biosynthesis of Prodyn peptides, it is presently unclear how morphine acts to increase these peptides. It is possible that morphine acts by a similar mechanism, activating mesostriatal dopamine neurons at the level of the substantia nigra and ventral tegmental area; however it is also possible that morphine acts quite differently. Thus, rather than increasing biosynthesis, morphine may, through feedback inhibition of Prodyn neurons, decrease release of peptides, leading to increases in intraneuronal stores. Studies are currently underway to determine the mechanism of action of morphine.

The qualitatively similar actions of morphine and amphetamine on striatal Prodyn peptides suggests that these peptides may play a role in the mechanism of action of these drugs of abuse. Although it is presently unclear what specific role Prodyn peptides might play, there are some intriguing possibilities. For example, since Prodyn peptides. in general, have a high selectivity for kappa opioid receptors, and since activation of kappa receptors is aversive, it is possible that the increased concentrations of these peptides seen after chronic drug administration may be involved in the aversive aspects of the withdrawal syndrome. On the other hand, since the shorter forms of Prodyn peptides. such as Dyn A 1-8, are potent agonists at mu and delta receptors, and since activation of mu and delta receptors is positively reinforcing, it is possible that Prodyn peptides may play a role in the euphorigenic properties of specific drugs. In support, we have previously obtained evidence suggesting that an acute injection of amphetamine may cause release of Prodyn peptides in the striatum, with much of the contribution coming from Dyn A 1-8. Further, it has been reported that opiate antagonists may interfere with the reinforcing actions of psychomotor stimulants. The present results appear to be very similar to those presented by G. Hanson and coworkers at this meeting. These investigators report increases in Dyn A, in both the caudate and the nucleus accumbens following repeated administration of cocaine, methamphetamine or phencyclidine. Thus it appears that a variety of drugs of abuse have interesting interactions with striatal Prodyn systems. These systems should be studied further to determine their specific role(s) in the actions of drugs of abuse, particularly in regards to the long-term consequences of drug use such as tolerance, dependence and withdrawal.

Supported by NIDA NRSA DA05336 to K.A.T.. and NIDA Grant DA02265 and NIMH Grant MH422251 to H.A.

AFFILIATION

Mental Health Research Institute, The University of Michigan
205 Washtenaw Place, Ann Arbor, Michigan 48109-0720

Modulation of Synaptosomal Free Intracellular Calcium in Naive and Morphine-Tolerant Mice: Correlation of Calcium Modulation In Vitro and In Vivo to Tolerance Development

Sandra P. Welch, Kirsten G. Olson, David L. Stevens and William L. Dewey

Calcium alterations in synaptosomes following morphine pretreatment were measured by the use of FURA-2 to determine whether alterations of free, intracellular calcium $[Ca^{++}]_i$ in synaptosomes occur with the acute administration of opiates in vitro and whether homeostatic alteration of free intracellular calcium concentrations upon chronic exposure leads to the development of tolerance. We also examined the effects of the calcium modulator, calcitonin gene-related peptide (CGRP) on the acute and chronic effects of morphine in vivo.

Synaptosomes were prepared from mouse brain using subcellular fractionation techniques and were loaded according to a procedure which facilitates hydrolysis of the cell permeant FURA-2/AM to the free FURA-2. The SPEX Cation Measurement System was used to measure the free calcium within the synaptosomes by use of FURA-2/AM. The free calcium concentration was determined using 224 nM as the KD for FURA-2. Each experiment was run in triplicate and repeated using at least 3 separate sets of synaptosomes. The synaptosomes were depolarized by the addition of a high KCl/Kreb's buffer such that the final depolarizing concentration of KCl in the sample cuvette was 50 mM.

Synaptosomes from naive, placebo-pelleted, and morphine pelleted animals were tested. These results indicate that synaptosomes from tolerant mice have significantly higher basal levels of intracellular calcium as well as significantly higher rises in intracellular calcium in response to a depolarizing stimulus.

Treatment	Ca Concentration	% change with depolarization
Naive	223(20) N=30	34 (3) % N=30
Placebo	285(36) N=16	30 (4)% N=16
Tolerant	385 (16)** N=24	52 (3)** N=24

** $p < 0.01$ from placebo

Synaptosomes from placebo and morphine-pelleted mice were tested with the calcium agonist BAY K 8644 to test for the activation of "L type" calcium channels in the tolerant versus the non-tolerant state. These results indicate that synaptosomes from tolerant mice are more sensitive to the activation of calcium channels than are synaptosomes from non-tolerant (placebo) mice, suggesting that an increase in the number or the activation of the L type calcium channel occurs in the development of tolerance to opiates.

CONCENTRATION OF BAY K 8644	% INCREASE IN BASAL CALCIUM (S.E.) Placebo	Tolerant
10^{-6}M	78 (23) n=6	200 (73)* n=6
10^{-7}M	25 (5) n=4	46 (10) n=6

*p< 0.01 from Placebo

In vivo testing CGRP was administered intrathecally (5 µl/ mouse) in saline vehicle. The chronic administration of CGRP (i.t.) was performed twice daily (2µg/ mouse per injection) for 7 days prior to testing with a challenge dose of morphine (4mg/kg, s.c.) in the tail-flick test. To evaluate the effects of CGRP on physical dependence to the opiates the animals were implanted with either a morphine or a placebo pellet at least 3 days prior to testing. The mice were challenged with various doses of naloxone (mg/kg, s.c.) alone or in combination with i.t. CGRP (2µg/mouse). The number of mice jumping from an 18 inch platform and the latency of the mice to jump was quantitated.

Chronically-administered CGRP (i.t.) attenuated the development of tolerance to morphine in morphine-pelleted mice. Placebo-pelleted mice challenged with a 4 mg/kg dose s.c. of morphine showed a significant 52% MPE in the tail-flick test as compared to the 2% MPE observed in morphine-tolerant mice. However, in the morphine-pelleted mice which received chronic CGRP for 7 days prior to the morphine challenge, the %MPE was 35% (S.E.=10), significantly different from those mice chronically injected with i.t. saline. CGRP enhanced the activity of low doses of naloxone on naloxone-precipitated withdrawal jumping in morphine-dependent mice, but did not precipitate jumping itself. The ED50 for naloxone (s.c.)-precipitated jumping was shifted from 0.1 mg/kg to 0.03 mg/kg when the naloxone was administered concurrently with the CGRP (i.t., 2µg/mouse). The latency of the mice to jump was reduced significantly by 50% with CGRP pretreatment in combination with naloxone.

The results in vitro are consistent with increased numbers of or the activation of calcium channels in opiate tolerant mice. The in vivo results indicate that homeostatic modulators of neuronal calcium such as CGRP may play a role in the development of tolerance and physical dependence to the opiates. Taken together the data indicate that the regulation of calcium concentrations in neurons in opiate-sensitive pathways plays an important role in tolerance and physical dependence.

AFFILIATION: Dept. of Pharmacology & Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0613

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In Vivo Binding of (+)-cis Methylfentanyl at the Opiate Receptor Complex and Behavioral Correlates: Evidence for a Novel Mechanism of Action

L. Band, V. Bykov, N. Greig, C.-H. Kim, A. Hauck-Newman, A. E. Jacobson, K. C. Rice and R. B. Rothman

Derivatives of 3-methylfentanyl are potent antinociceptive a'gents and produce morphine-like catalepsy (Xu et al. 1987; Zhu et al. 1983). (+)-Cis-methylfentanyl (MF), an analog of the selective delta opioid acylating agent SUPERFIT, displays wash-resistance in binding to the opiate receptor complex *in vitro* (Rothman et al., in press). In the present study, the *in vivo* binding and behavioral efficacy of this compound were examined in relation to the receptor complex.

IN VIVO BINDING AND BEHAVIORAL MEASURES

Male Sprague-Dawley rats (250-300 g) were injected intravenously with 50 µg/kg of MF or saline. Thirty min after MF injections, two groups of animals were injected with (+)- or (-)-6-desoxy-6-beta-fluoro-naltrexone (cycloFOXY), an opiate antagonist. At 120 min after the initial injection, animals were tested behaviorally. Catalepsy was determined by measuring: 1) the latency to remove the forelimbs from a bar suspended 9 cm above a horizontal surface (bar test], and 2) the latency to remove the right hindlimb from a platform 2 cm in height. A maximum score of 120 s was assigned for each test. Behavioral tests were followed by decapitation and quick freezing of brains in -80°C isopentane. Brain tissue was polytroned in Tris buffer, pH 7.0, at 0°C, followed by centrifugation and pouring off of the supernatant (wash); the wash procedure was repeated and membranes were frozen. Assays for tritiated [D-ala^2,D-leu^5]enkephalin ([^{3}H]DADL) and (3,14-dihydroxy-4,5-alpha-epoxy-6-beta-fluoro-17-methylmorphinan) ([^{3}H]FOXY) binding were conducted as previously described (Rothman et al. 1987, 1988). [D-pen^2, L-pen^5] enkephalin (DPLP) revealed the delta binding site of the receptor complex (delta$_{cx}$); [^{3}H]FOXY labeled mu sites.

RESULTS AND CONCLUSIONS

Both measures of catalepsy yielded identical results. Intravenous administration of MF produced maximal catalepsy and reduced binding at all labeled sites. (-)-, but not (+)-, cycloFOXY, completely reversed catalepsy and attenuated masking of all sites labeled by [3H]DADL ($p < .05$), including delta,, sites ($P < .001$). Mu sites were not recovered.

Effects of (+)-CIS-methylfentanyl on [^{3}E]DADL and [^{3}E]FOXY binding and catalepsy

Condition	Specific Binding (fmol Protein)	Specific Binding (% of Control)	Bar Test (Seconds)
[^{3}H]DADL, total			
SALINE	119.60 ± 12.61	100	1.25 ± 0.25
MF	57.44 ± 2.32	48.03 ± 1.94*	120.00 ± 0
MF, (-)-cyFOXY	77.64 ± 5.60	64.92 ± 4.68*†	1.25 ± 0.25
MF, (+)-cyFOXY	60.83 ± 4.37	50.86 ± 3.65*	120.00 ± 0
[^{3}H]DADL, delta$_{cx}$			
SALINE	44.24 ± 4.50	100	1.25 ± 0.25
MF	12.25 ± 0.57	27.69 ± 1.29*	120.00 ± 0
MF, (-)-cyFOXY	23.93 ± 1.99	54.10 ± 4.50*††	1.25 ± 0 25
MF, (+)-cyFOXY	13.01 ± 1.10	29.41 ± 2.49*	120.00 ± 0
[^{3}H]FOXY			
SALINE	89.05 ± 7.08	100	1.25 ± 0.25
MF	25.00 ± 1.94	28.07 ± 2.18*	120.00 ± 0
MF, (-)-cyFOXY	35.39 ± 4.23	31.22 ± 5.83*	1.25 ± 0.25
MF, (+)-cvFOXY	24.06 ± 2.73	27.92 ± 3.07*	120.00 ± 0

Data points are mean values ± S.E.M.(n = 4); *p < . 01, as compared to saline; †p < .05, ††p <.01, as compared to MF. Data were analyzed using t-tests for independent samples.

These data suggest that MF acts at delta,, in producing catalepsy and may act at delta sites outside the receptor complex. Injection of (-)-cycloFOXY at 20 min vs. 90 min prior to collection of brains results in full (data not shown) rather than partial recovery of delta$_{cx}$ sites. Slow release of MF from lipid tissue or plasma platelets may underlie both the partial recovery of sites and long duration of behavioral effects. The complete‘ reversal of behavioral measures accompanying partial unmasking of delta,, sites (54% of control levels) may indicate that occupancy of a critical ratio of these receptors is necessary to produce catalepsy.

AFFILIATIONS

[1]Unit on Receptor Studies, LCS, NIMH, [2]Laboratory of Neurosciences, NIA, [3]Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892

NOTE: References will be made available on request.

Biological Evaluation of Compounds for Their Physical Dependence Potential and Abuse Liability. XIII. Drug Testing Program of the Committee on Problems of Drug Dependence, Inc.

Arthur E. Jacobson

TESTING PROGRAMS OF THE CPDD

The Drug Testing Program of the Committee on Problems of Drug Dependence (CPDD) relies upon researchers in three universities and NIH to obtain data on the physical dependence potential and abuse liability of analgesics, and of stimulants and depressants. The examined compounds are obtained from three distinctly different groups, university researchers, pharmaceutical companies, and national and international governmental agencies. The data which are needed for these groups are different, and have been noted (Jacobson 1987, May and Jacobson 1989). The Drug Testing Program receives advise and guidance from, and in general reports to the Animal Testing Committee (Dr. T. J. Cicero, Chairman) of the CPDD.

Animal Testing Committee

The Animal Testing Committee is composed of CPDD members with an interest and expertise in the Animal Testing area and expert consultants who have been appointed to the group (Drs. Steven Holtzman. Thomas Burks, Theodore Cicero, James Woods, William Woolverton, Graham Patrick, Gail Winger. Joseph Brady, Harold Kalant, Kenner Rice, Jack Mendelson. William Dewey, Louis Harris, Martin Adler, and Arthur Jacobson). Nine of these fifteen individuals are not, presently, members of the Board of the CPDD. and four of the nine have never been members of the CPDD. The Animal Testing Committee met jointly on May 8-9, 1989. in Ann Arbor with the Human Testing Committee (co-chaired by Drs. Nancy Mello and Marian Fischman), and the work of the testing groups was discussed.

Communication

Results of the work from the two testing programs, performed by researchers at various universities and NIH, are communicated to Dr. Cicero's Animal Testing Committee differently. The groups concerned with testing drugs as opioids are at the University of Michigan (UM) (Drs. James A. Woods, Fedor Medzihradsky, Charles Smith, Gail Winger.

and Charles France), the Medical College of Virginia (MCV) (Drs. Mario Aceto, Edward Bowman, Louis Harris, and Everette May), and the National Institutes of Health (NIH), NIDDK (Dr. Arthur E. Jacobson and Mariena V. Mattson).

The reports which I receive during the year from the groups involved with the opioid program are forwarded so that each group is aware of the work which has been completed. Their work is related to the Animal Testing Committee at a meeting a month prior to the annual meeting of the Board of Directors of the CPDD. Data from these laboratories are sent. by me, to the submitters of the compounds without prior review by the Animal Testing Committee, but after discussion of the MCV work at quarterly meetings between the groups at MCV and NIH (with a representative from the National Institute on Drug Abuse (NIDA)). or after perusal of the results from UM both at UM and NIH. The testing is done under a coded NIH number in a "blind" fashion by the university groups and they become aware of the chemical structure of the drugs only after I have obtained the submitter's release for the data, and that occurs, generally, in March or April. The data released for publication by the submitter were discussed at the Animal Testing Committee meeting in May. 1989.

The work on stimulants and depressants under the auspices of the CPDD is done similarly, under a CPDD number. The groups involved are at the University of Chicago (Drs. William Woolverton and Michael Nader), MCV (Drs. Graham Patrick and Louis Harris), UM (Drs Gail Winger and James Woods), and NIH (Dr. Arthur Jacobson). The reports from the stimulant/depressant groups are handled differently than those from the opioid testing groups. Prior to sending the results to the submitter of the drug, complete data on the drug are distributed to members of the Animal Testing Committee for their comments, and these comments are incorporated into my letter to the submitter of the drug, with the data from the various laboratories. The results, then, are sent with the imprimatur of the CPDD.

The Animal Testing Committee has not been made aware of most of the structures of the stimulants and depressants examined this year since the data on four of the seven examined compounds have not, as yet. been released by the submitter. The (first) overall report on the examined stimulants and depressants to the Animal Testing Committee in May contained experimental data on all of the compounds evaluated and both structural information and partially completed experimental data on the compounds evaluated for the World Health Organization (WHO). The work for the WHO was only partially completed due to time constraints, and the current system of prioritization. These data have not. as yet. been officially sent to the WHO, but preliminary findings were given to Dr. L. Harris, a CPDD member and a member of the WHO Expert Committee, for transmittal and discussion at the Expert Committee meeting in Geneva, in April, 1989.

We have, with this testing program, completed our initial nine months from the time the program was publicly opened (to pharmaceutical companies). Compounds from universities have not,

as yet. been accepted into the program because this program has been completely funded by the CPDD and it is quite expensive.

The CPDD is indebted to the university researchers who cooperate with the CPDD and make their work with the opioids and the stimulant/depressants available to us.

Possible Expansion of the Drug Testing Program

Initial discussions have' been held between groups at NIH and UM and the Chairman of the Animal Testing Committee on the feasibility and desirability of including another class of abused drugs in the Drug Testing Program, drugs acting at phcncyclidinc (PCP) binding sites in the brain. PCP is one of the most widely abused drugs in several major U.S. cities, especially Washington, D.C. Since it is relatively inexpensive and easily available through synthesis in clandestine laboratories, few, if any, controlled substance analogs based on PCP have appeared for illicit use. Interest in PCP-like compounds, then, is based on its mode of action in the brain and the potential medical usefulness of PCP analogs. These analogs have been noted to be useful for, among other things, the treatment of ischcmia (Iversen et al. 1988) and, possibly, epilepsy (Rogawski et al. 1989). The PCP analogs which are sought for their medical utility are those, of course, which lack the psychotomimetic effects of PCP. The PCP-like drugs which are likely, eventually, to be introduced by pharmaceutical companies for human use might well need testing for their PCP-like abuse liability before introduction to the public. The laboratories at UM and at NIH, NIDDK, arc currently involved with research on PCP-like drugs, and could be initially utilized if this testing is found to be necessary and desirable. Other laboratories might also be interested in joining this program if a sufficient number of PCP-like drugs become available for abuse liability testing.

Database for Opioids

Over the past 35 or so years a considerable amount of data has been generated by the laboratories associated with the Drug Testing Program of the CPDD on opiates and opioid-like compounds. These data have been printed, “in the various proceedings of the CPDD meetings as ‘minutes’ through 1968, as ‘reports’ through 1974 and finally as ‘proceedings’ in 1975 and 1976, when NRC relinquished sponsorship. The 1977 and 1978 proceedings were published by the CPDD, Inc. Starting in 1979, the proceedings have been published as part of the Research Monograph Series of NIDA and are archival. Before 1979 they were labeled non-archival” (May and Jacobson 1989). Complete compilations are maintained at NIH, MCV and UM. However, for outside groups and for researchers interested in obtaining information on opioids, a significant part of these data have been unavailable. A computerized list of the examined compounds, cross-indexed by NIH number, submitter, company code number, chemical family, etc., was initiated at NIH and is maintained and updated annually at UM, with the aid of Dr. May at MCV. This index can be used to find the year of the publication in which the

original work on the compound can bc located. Unfortunately, this docs not help those rcscarchcrs who do not have access to the complete set of data in the various volumes noted above. Further, the availability of the computerized index and the complete data set would not make the task of correlating the chemical structures of opioids with their biological activity, or starching for compounds with specific biological activities, less laborious. Ii is conceivable that computerization of the biological data combined with the chemical structures of the opioids which have been examined would allow much more facile entry into the database and allow researchers to ask questions which would otherwise be impossible to resolve.

Within the past several years Dr. J. Woods at UM has initiated the formation of a computerized database of the biological information which has been gathered under the auspices of the CPDD at UM, MCV, and NIH. The data which have been computerized lacks chemical structural information relating to the compounds which were evaluated. Several software packages are currently commercially available for different computer systems (Apple and IBM) which allow the more-or-less facile entry of stereochemically correct chemical structures. No software is available, that I am aware of, for the Apple computer system which allows retrieval of information based on part structures. There is at least one software package which can be used for this purpose on IBM-type (MS-DOS or OS/2 operating system) computers. This software, from Molecular Design Ltd. (ChemBase). is available, but it is expensive and, perhaps, not as easily utilized as, for example. ChemDraw for the Apple Macintosh computer. A computer which facilitates multi-tasking, the simultaneous, or nearly-simultaneous display and interaction of several commercially available software programs of diverse types. would be most desirable to input data, query the database and store gained information in new data subsets. At this time we are forced to eliminate the Apple computer system because of the lack of suitable chemical drawing software allowing part- structure retrieval, and the MS-DOS operating system is not easily utilized as a multi-tasking system. The IBM OS/2 and the UNIX system were designed as multi-tasking systems, but the software now available for either operating system is inadequate for our purposes.

The desirability of the computerization of the database, and the available software and computer systems were discussed with the Animal Testing Committee in May, 1989. This Committee suggested that attempts should be made to begin the compilation of chemical structures and biological data using the Molecular Design Ltd. ChemBase software and an available MS-DOS operating system, with an appropriate subset of data. The chemical structures would be introduced into a database at NIH. Implementation of the suggestion will be attempted when funding is available for personnel, software. et al. Other commercial software packages will, also, be examined for their utility for these purposes.

STATISTICS

About 55 compounds were tested at MCV in the opioid program this year (Aceto et al. 1990). and results on 40 compounds investigated at UM are included in the rcpon from UM (Woods et al. 1990). These 95 reports concern 68 compounds of various structural types (tables 1-9). The compounds were obtained from university researchers, pharmaceutical industry, and governmental groups.

Seven compounds were received for testing as stimulants or depressants, and these were obtained from pharmaceutical industry or governmental groups. Results obtained on the three depressants obtained for examination for the WHO have been briefly noted in this report.

THE OPIOID PROGRAM

The chemical structures of the various compounds which were tested as opioids in 1989 are listed in Tables 1-9, together with the biological work which was done with them. The compounds which were examined have been placed according to their conventional chemical classification (i.e. 4,5-epoxymorphinans in tables 1-3, 6,7-benzomorphans in table 4, 4-phenylpiperidines in tables 5-6, fentanyl-related compounds in table 7, and miscellaneous compounds in tables 8-9.

Stereochemical Effects

It is convenient to view the tested compounds according to their chemical classification. Generally, most newly synthesized compounds which have the basic chemical structure known to interact with opioid receptors (and the classification scheme for the structures in the tables denote a considerable number of the major classes of those which have been found to do so) will have some opioid activity. Within a particular class, of course, there are important factors which dictate activity. One of these factors relate to the stereochemistry of the molecule. The enantiomers of racemic mixtures which have been found to have opioid activity are generally the (-)- (levo) enantiomers.

Racemic mixtures and enantiomers of the totally synthetic opioids have, historically, been obtained and examined, and examples of these can be seen in table 4. The (+)-6,7-benzomorphans have little or no opioid activity, compared with their (-)-enantiomeric relatives. They may be of interest, however, for other reasons. Until the last few years there were no (+)-enantiomers available for those analgesics or antagonists which had been prepared more-or-less directly from a plant product (e.g., analgesics or antagonists in the 4,5-epoxymorphinan series, which includes morphine, etorphine, codeine, naloxone, naltrexone, etc.). It was not, then, at all necessary to state whether the morphine or codeine used in an experiment was (-), since no other form, nor the racemate, existed. Recently however, Rice (1985) reported a practical total synthesis of this class

of opioids. and several of the more important dextro enantiomers of the 4,5-epoxymorphinans have now become available in sufficient quantity for biological testing ((+)-morphine, (+)-codeine, (+)-etorphine, etc.). Three of the compounds shown in table 3 resulted from this practical total synthesis (NIH 10617, 10620, and 10621).

The 4.5-Eooxymorphinans

(-)-Etorphine is one of the most potent opioid agonists in vivo. Although its binding to opioid receptors is not completely in accord with its potency in vivo, it is obvious that exceptional stereoselectivity is shown by the enantiomers of etorphine, in vivo and in vitro. In table 3, (+)-etorphine can be seen to be ineffective in displacing the [^{3}H]-(-)-etorphine radioligand from opioid receptors at concentrations >10 µM. The ca. 10,000 fold binding stereoselectivity between the dextro and lcvo enantiomers of etorphine is remarkable. In 1964, at UM, (-)-etorphine (NIH 8068) was found to be equivalent to morphine, in a single dose suppression (SDS) study in monkeys, at a dose of 0.0012 mg/kg. The (+)-etorphine (NIH 10617) was studied in SDS at MCV this year and was found to be inactive at 8 mg/kg. The (-)-enantiomer, in vivo, was thus more than 6666 times more potent as an opioid than (+)-etorphine. An interesting by-product of this stereoselectivity is that the chemical resolution or separation of these enantiomers can be more accurately checked in vitro or in vivo than it can be by chemical instrumentation.

The (+)-thevinone and (+)-19-propylthevinol (NIH 10620 and 10621, in table 3) synthesized by Grayson and Rice (1990) are, like (+)-etorphine, inactive as opioids. It is interesting to note that although the former (NIH 10620) docs not interact at opioid receptors, it appears to have significant opioid antagonist activity in the electrically stimulated mouse vas deferens (VD) preparation. It is unusual to find opioid antagonists which do not interact with CNS opioid receptors and this work will, therefore, be repeated. The in vivo data from MCV will be reported next year, but sufficient work has been done with these compounds thus far to say that they are both inactive as opioids in vivo (to 16 mg/kg in the SDS assay).

A Non-Phenolic 4,5-Epoxymorohinan and N-Methyl Substituted Antagonists

Although most 4,5-epoxymorphinans without a phenolic hydroxyl group in the C-3 position on the aromatic ring are relatively inactive, NIH 10501 was found to be as potent as, or more potent than morphine in antinociceptive assays last year (Aceto et al. 1989). and has been found this year (table 1) to have activity in vitro as well.

The cyclopropylmethyl substituent on nitrogen in this class of opioids is known to confer narcotic antagonist properties to the molecule. the comparable N-methyl relative having agonist effects. Several exceptions have been found previously, and again this year. NIH 10544 and 10594 (table 1). which have an N-methyl moiety, do not substitute for morphine in the SDS assay and both of them were noted

to have antagonist activity in the TFA assay. They were both found to be selective mu opioid antagonists in the VD preparation. with NIH 10544 having the potency of naloxonc. However, saturation of the double bond in the cinnamoylamino moiety induces a qualitative change in activity, from antagonist to agonist. Thus NIH 10545, the saturated relative of NIH 10544, was found to have selective mu agonist activity in the VD preparation (the agonist activity was also found in vivo data from MCV), in contrast with the potent and selective mu antagonist activity of NIH 10544.

Other compounds in the C-14 substituted cinnamoylamino series (e.g., NIH 10443 and 10445) were discussed last year, and some of those which had the N-cyclopropylmethyl substituent were found to be very long acting, and potent narcotic antagonists.

A Potent 4,5-Epoxymorphinan, and Selective Delta Opioid Receptor Antagonists

The most potent of the 4,5-epoxymorphinans which were examined this year was NIH 10549. It was found to be more than 700 times as potent as morphine in the PPQ assay and 290 times more potent than morphine in the TF assay. Three compounds which were originally reported by Portoghese et al. (1988), were resynthesized elsewhere and submitted to us for testing. These compounds, NIH 10589, 10590 and 10591, exacerbate withdrawal in the SDS assay and two of them were potent delta selective antagonists in the opioid receptor assay (NIH 10589 and 10590). The NIH 10591, which has an N-methyl substituent, acted as an agonist in the VD preparation, but not in vivo. These compounds show great promise for the examination of the specific biological effects of delta agonists and antagonists.

6,7-Benzomorphan Narcotic Antagonists

The raccmate and enantiomers of a 6,7-benzomorphan with an N-methylallyl substituent were examined (NIH 10595, 10608 and 10612 in table 4) and the levo enantiomer (NIH 10595) was found to have agonist-antagonist activity. Its duration of action in nonwithdrawn monkeys was found to be much longer than naloxone. The racemic N-pentyl benzomorphan (NIH 10573 in table 4) was reevaluated this year. When it was examined in 1977 (as NIH 7785, tested from 2 to 16 mg/kg). it was found to only partially reverse abstinence in the SDS assay. However, it was not tested for its possible antagonist activity at that time. The compound was found, this year, to be an agonist-antagonist at MCV. It precipitated withdrawal in non-withdrawn monkeys. The antagonist activity of NIH 10573 was not observed in the tail flick antagonist assay vs. morphine (TFA) or in the VD preparation.

Extremely Potent Compounds Related to Fentanyl

Although not quite as potent as carfentanil (NIH 10570 in table 7) was noted to be last year, NIH 10579 in table 7 was found to be more than 3000 times more potent than morphine in the PPQ assay. Many

fentanyl-like compounds have, in the past, been submitted to us from governmental groups. These drugs had been found to have been prepared illicitly and marketed illegally, and were sent to us for the purpose of obtaining sufficient pharmacological data to enable their scheduling. There are, also, several pharmaceutical and university groups interested in this class of opioid. Carfentanil, for example, was tested under CPDD auspices prior to its being marketed for use as an animal tranquillizer.

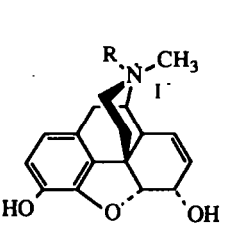
Compounds with Non-classical Opioid Structures

Most of the miscellaneous compounds in tables 8 and 9 have little or no opioid activity. lbogaine (NIH 10567 in table 9) was sent to us for examination with the idea that it might have potential as a preventative for cocaine and narcotic abuse. Extracts of the lboga plant, from which ibogaine is obtained, have been noted to have some rather peculiar properties (Merck Index, # 4793). These extracts are said to have been used "by African natives while stalking game, to enable them to remain motionless for as long as two days while retaining mental alertness". The compound was found, at MCV, to have weak antinociceptive activity only in the PPQ assay, nor does it interact with opioid receptors in rat brain homogenates or in the VD preparation with any effectiveness. It partially substitutes for morphine (at 2 or 8 mg/kg) in the SDS assay, but this is not necessarily indicative of opioid-like activity. We will examine the compound further, next year, using rat infusion assays if a sufficient quantity of the pure compound can be obtained. It is likely that most of the compounds shown in tables 8 and 9 were not synthesized as centrally acting analgesics.

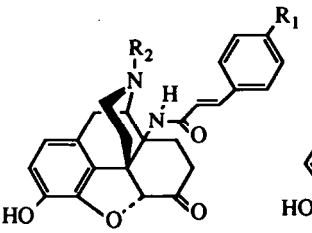
In order to summarize the biological work from UM (Woods et al. 1990). and MCV (Aceto et al. 1990). in a few tables, a considerable number of abbreviations have been used. These are listed after table 9. Only that work which was actually done this year has been recorded in the tables. Previously reported work on compounds is noted with the year in which the report appeared. It should be emphasized that the year noted is the titled year (e.g.. Problems of Drug Dcpendcnce. 1986). not the year of actual publication (which usually, but not always, occurred during the year following that denoted in the title of the publication).

Thus, from these tables, the reader can see all of the work done this year, or when some of the work on the compound was previously done, across all facilities associated with the opioid program under the auspices of the CPDD. If the reader would like further, or more complete information on a particular compound, the original report of Woods et al. (1990) and Aceto et al. (1990) should be perused through use of the assigned NIH number, since all reports are cross-referenced by NIH number.

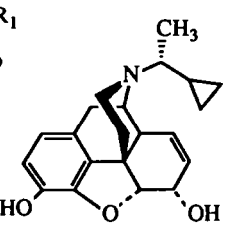
TABLE 1. 4,5-EPOXYMORPHINANS[a]



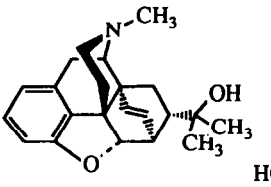
9994: R=CPM
9995: R=CH_3



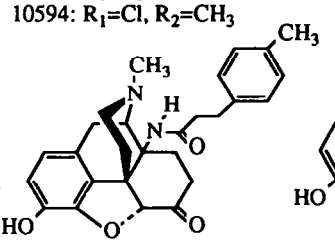
10443: R_1=Cl, R_2=CPM
10445: R_1=Br, R_2=CPM
10544: R_1=R_2=CH_3
10594: R_1=Cl, R_2=CH_3



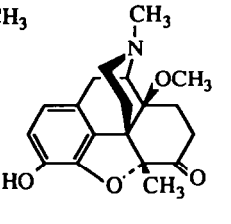
10497



10501



10545

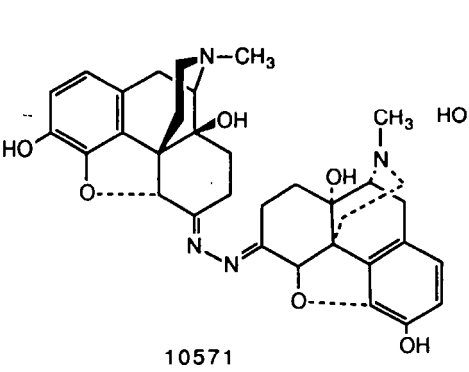


10549

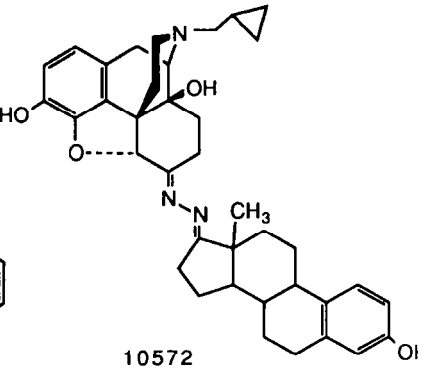
	MOUSE ED50/AD50				IN VITRO		MONKEY	
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	OTHER
9994	I[b]	11.9[b]	I[b]	I[b]	49.3	2.8E-5(46)[A]	-	-
9995	I[b]	I[b]	I[b]	I[b]	2750	2.8E-7(55)[NA]	NS(5.6,10)[b]	-
10443	I[c]	I[c]	I[c]	0.12[c,d]	0.65[e]	NE[e,f]	NS(0.05)[c,g]	NOTE[h]
10445	I[c]	7.1[c]	I[c]	0.8[c,d]	0.8[c]	NE[c,i]	NS(0.4,1.4)[c,j]	NOTE[h]
10497	-	0.03	2.0	I	2.14	4.7E-8(74)[NA][k]	CS(0.75,3)	NOTE[l]
10501	-	0.2[e]	0.05[e]	I[e]	291	4.8E-7(100)[A]	-	-
10544	-	I	I	0.6	0.64	NE[i]	NS(4,12)	-
10545	I	0.09	0.5	I	0.51	6.6E-9(74)[A][m]	CS(1)	-
10549	-	0.0003	0.02	I	-	-	-	-
10594	-	1.2	I	3.8	0.3	NE[i]	NS(2,8)	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) PREVIOUSLY REPORTED - 1983. c) PREVIOUSLY REPORTED - 1987. d) LONG DURATION OF ACTION. e) PREVIOUSLY REPORTED - 1988. f) POTENT, "IRREVERSIBLE" ANTAGONIST OF μ, δ, κ-RECEPTORS. g) PRECIPITATED WITHDRAWAL. h) DURATION OF ANTAGONISM OF MORPHINE. i) SELECTIVE μ-ANTAGONIST. j) SEVERE WITHDRAWAL NOT RELIEVED BY NALOXONE. k) κ-AGONIST IN MVD. 1) MONKEY ANALGESIA (ED50=1.7); SI=IN; DD-GENERALIZED TO CODEINE (0.01, 0.001). m) SELECTIVE μ AGONIST.

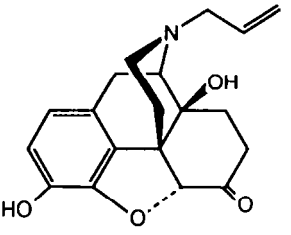
TABLE 2. 4.5-EPOXYMORPHINANS (CONTINUED)[a]



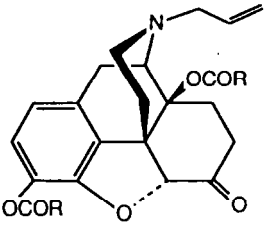
10571



10572



10562, 7890: NALOXONE

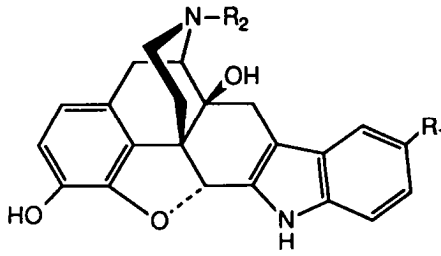


10605: R=CH3
10606: R=C2H5

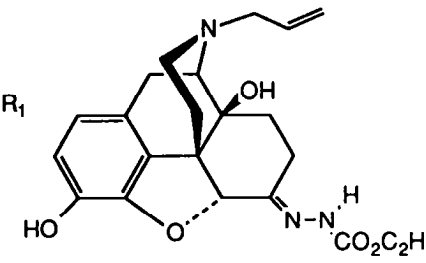
	MOUSE ED50/AD50				IN VITRO		MONKEY	
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	
10562	-	I[b]	I[c]	0.03[c]	6.26[c]	NE[c,d]	NS[e](0.0125,0.05)	
10571	-	0.03	0.09	I	40.2	5.8E-7(99)[A]	CS[e](0.25,1)	-
10572	-	I[c]	I[c]	0.03[c]	1.69	INSOLUBLE	-	
10605	-	-	-	-	11.8	NE[f]	-	-
10606	-	-	-	-	59.2	NE[g]	-	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) REPEATED PPQ. c) PREVIOUSLY REPORTED - 1988. d) ANTAGONIZED μ, δ, κ-AGONISTS. e) EXCEPT FOR WET DOG SHAKES. f) ANTAGONIST, LESS POTENT THAN NALTREXONE. g) SELECTIVE μ RECEPTOR ANTAGONIST.

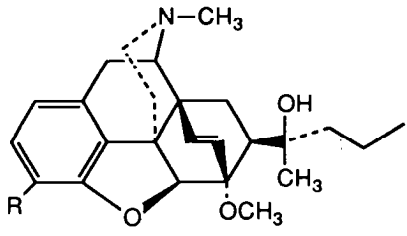
TABLE 3. 4,5-EPOXYMORPHINANS (CONTINUED)[a]



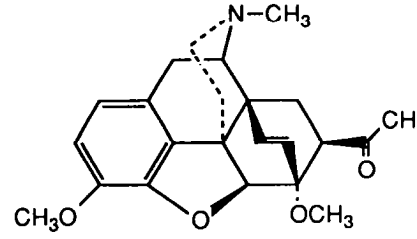
10589: R_1=H, R_2=CPM
10590: R_1=CH_3, R_2=CPM
10591: R_1=H, R_2=CH_3



10607



10617: R=OH ((+)-ETORPHINE
10621: R=OCH_3 (+)

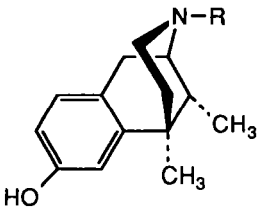


10620: (+)

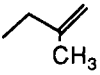
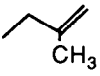
	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10589	-	I	I	I	μ=9.53[b]	SE[c]	NS[d](3,12)
10590	-	I	I	I	μ=6.42[e]	NE[c]	NS[d](3,12)
10591	-	I	I	I	μ=10.8[f]	4.5E-8(61)[A]	NS[d](3,12)
10607	-	-	-	-	7.2	1E-6(32)[A][g]	-
10617	-	I	I	I	>10μM	NE	NS(2,8)
10620	-	-	-	-	>10μM	NE[h]	-
10621	-	-	-	-	>10μM	NE	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) δ_1=0.214. κ=20.5. c) SELECTIVE δ RECEPTOR ANTAGONIST. d) EXACERBATED WITHDRAWAL. e) δ_1=0.204. κ=9.01. f) δ_1=3.37. κ=688. g) PARTIAL AGONIST WITH SIGNIFICANT ANTAGONIST ACTIVITY. h) NON-SELECTIVE OPIOID ANTAGONIST.

TABLE 4. 6,7-BENZOMORPHANS[a]



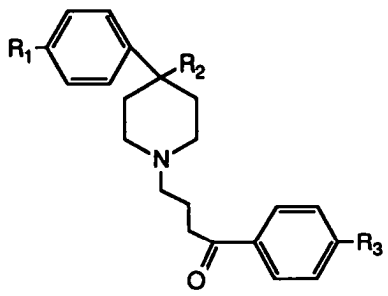
7589: R=*n*-BUTYL (±)
7912: R=ALLYL (±)
8773: R=ALLYL (-)
8775: R=ALLYL(+)
10168: R=CH_2-CH=CH-Cl (-)
10556: R=*n*-PROPYL (+)
10564: R=CH_2-CH_2-CO_2H (±)
10565: R=*n*-BUTYL (+)
10566: R=*n*-BUTYL (-)

10569: R=*n*-PENTYL (-)
10573: R=*n*-PENTYL (±)
10595: R=  (-)
10608: R=  (+)
10612: R=  (±)

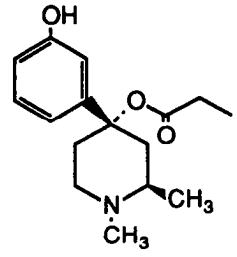
	MOUSE ED50/AD50 IN VITRO						MONKEY	
NIH#	HP	PPO	TF	TFA	RBH	VD	SDS	NW
7589	I[b]	-	-	-	101	NE[c]	-	PW[b]
7912	I[b]	10.4	I	1.9[d]	-	-	-	PW[b]
8773	I[e]	1.3	I	0.2[f]	-	-	NS[e]	PW[e](0.013-0.05)
8775	I[e]	I	I	13.2	-	-	NS[e,g]	-
10168	I[h]	1.7[h]	I[h]	0.004[h]	2.72	2.6E-8(45)[SA]	NS[h]	PW[h]
10556	I[i]	I[i]	I[i]	I[i]	>10µM	NE[i,j]	PS(2,8)	-
10564	I[i]	I[k]	I[i]	I[i]	>10µM	NE[i]	-	-
10565	-	I	I	I	>10µM	NE[i,l]	PS(3,12)	-
10566	-	I[i]	I[i]	I[k]	47.2[i]	3E-7(64)[A][i]	PSi(2,8)	-
10569	I[i]	0.2	1.3	I	102[i]	7E-7(100)[A][i]	CS(1,4)	-
10573	-	0.2	0.8	I	173	1E-6(100)[A]	PS[m](2,8)	PW(2,8)
10595	-	0.8	I	0.8	77.6	2.7E-7(51)[SA][n]	NS[o]	PW(0.025,0.1)[p]
10608	-	18.6	I	I	>6µM	NE	NS(2,8)	-
10612	-	1.8	I	0.9	143	NE[c]	NS[o]	PW(1.2)[0.04N]

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) PREVIOUSLY REPORTED - 1972. c) OPIOID ANTAGONIST. d) AD50 OF 7912 VS. ED50 OF MORPHINE=3.3. AD50 OF NALOXONE AND YOHIMBINE VS. ED80 OF 7912=3.4 AND 0.4, RESPECTIVELY. e) PREVIOUSLY REPORTED - 1973. f) AD50 OF 8773 VS. ED50 OF MORPHINE=1.2. AD50 OF NALOXONE AND YOHIMBINE VS. ED80 OF 8773=0.13 AND 0.12, RESPECTIVELY. g) CNS DEPRESSION NOT REVERSED BY NALORPHINE. h) PREVIOUSLY REPORTED - 1983. i) PREVIOUSLY REPORTED - 1988. j) WEAK ANTAGONIST OF µ AND κ-RECEPTORS. k) REPEATED THIS YEAR. I) WEAK ANTAGONIST OF µ,δ, κ-RECEPTORS. m) EXCEPT FOR WET DOG SHAKES. n) µ AGONIST, AND ANTAGONIST. o) EXACERBATED WITHDRAWAL. p) MUCH LONGER DURATION OF ACTION THAN NALOXONE (0.01N).

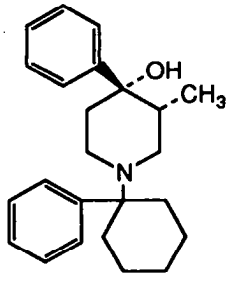
TABLE 5. 4-PHENYLPIPERIDINES[a]



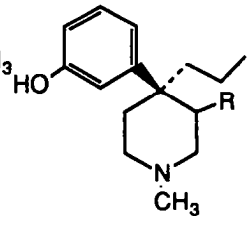
8032: R_1=Cl, R_2=OH, R_3=F (HALOPERIDOL)
10495: R_1=R_3=H, R_2=OCOC2H5



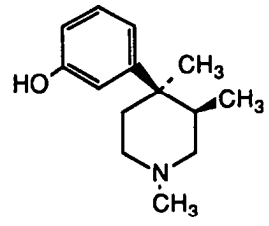
10526



10553 (+)
10554 (-)



10596: R= ···CH3
10597: R= ◄CH3

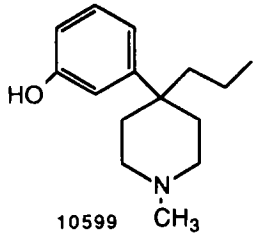


10598

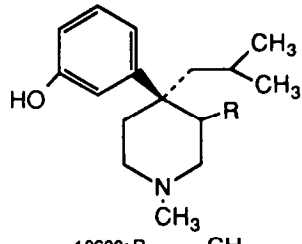
	MOUSE ED50/AD50				IN VITRO		MONKEY	
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	OTHER
8032	-	0.01[b]	14.6[b,c]	I[b]	-	-	NS(0.005-0.08)[d]	RI-SM[e]
10495	0.3[b]	0.07[b]	0.3[b]	I[b]	30[b]	5E-8(99)[A][b]	CS(0.1)[b]	RI-SM[f]
10526	I[b]	3.7[b]	I[b]	I[b]	2885	5E-8(29)[NA]	-	-
10553	I[b]	0.7[b,g]	8.7[b]	I[b]	-	-	-	-
10554	I	2.5	I	I	-	-	-	-
10596	-	1.4	I	I	-	-	-	-
10597	-	2.9	I	6.1	1026	NE[h]	-	-
10598	-	I	I	1.2	403	NE[i]	-	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) PREVIOUSLY REPORTED - 1988. c) ERRATIC. NALOXONE PRODUCED 65% ANTAGONISM VS. ED60 OF NIH 8032. d) REPORTED ALSO IN 1963. e) NS (0.003 mg/kg/day); RI-PPD: MORPHINE PLUS NIH 8032 QUALITATIVELY SIMILAR TO MORPHINE ALONE. QUANTITATIVE CHANGE NOTED. f) PS (20); RI-PPD: MORPHINE-LIKE. g) AD50 OF NALOXONE VS. ED80 OF NIH 10553 = 0.06. h) NON-SELECTIVE ANTAGONIST. i) ANTAGONIST WITH SLIGHTLY HIGHER AFFINITY FOR μ AND κ THAN δ.

TABLE 6. 4-PHENYLPIPERIDINES (CONTINUED)[a]

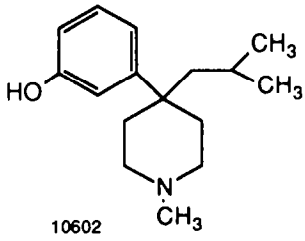


10599

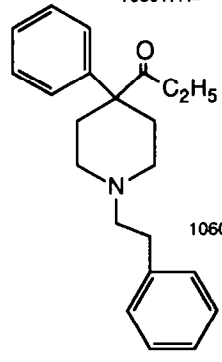


10600: R= ---CH_3

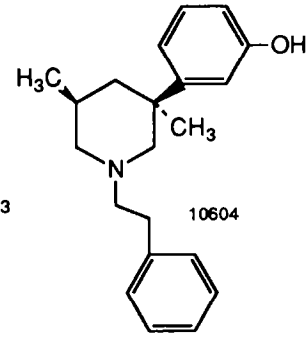
10601: R= ◀CH_3



10602



10603



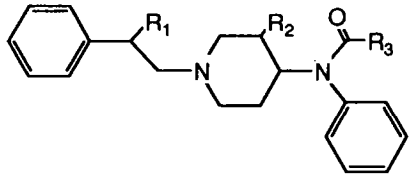
10604

NIH#	HP	PPQ	TF	TFA	RBH	VD
10599	-	0.4	0.5	I	146	5.6E-7(91)[A]
10600	-	0.2	2.3	I	46.3	1.3E-7(32)[A]
10601	-	1.7	17.6	I	1743	NE[b]
10602	-	0.3	0.8	I	-	-
10603	-	6.3	6.4	I	1399	NE[c]
10604	-	4.7	I	I	-	-

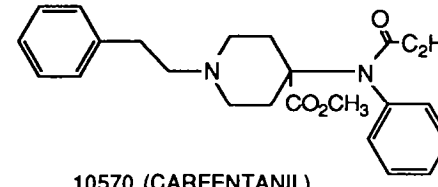
MOUSE ED50/AD50 IN VITRO

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) NON-SELECTIVE ANTAGONIST. c) OPIOID ANTAGONIST, LESS POTENT THAN NALTREXONE.

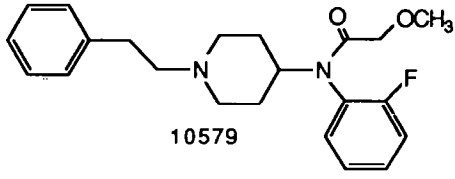
TABLE 7. FENTANYL-RELATED COMPOUNDS[a]



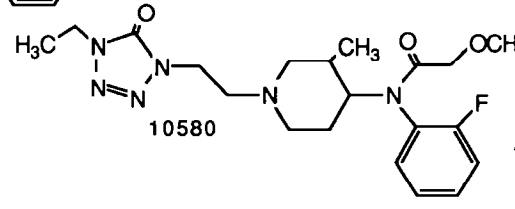
10551: R_1=OH, R_2=CH3, R_3=ETHYL
10576 (10488): R_1=R_2=H, R_3=*n*-BUTYL*



10570 (CARFENTANIL)



10579



10580

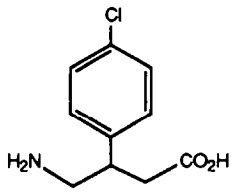
NIH#	HP	MOUSE ED50/AD50 PPQ	TF	TFA	IN VITRO RBH	VD	MONKEY SDS
10551	<0.0003[b]	0.00013[b]	0.0002[b,c]	I[b]	4.42[b]	4E-10(98)[A][b]	CS [25000xM]
10570	<0.0004[b]	0.00006[b]	0.0002[b,d]	I[b]	72[b]	1E-10(97)[A][b]	CS [25000xM]
10576	7.5[e]	2.4[e]	5.4[e]	I[e]	436[b]	NE[f]	CS(5.0)[e]
10579	-	0.000075	0.002	I	49.2	1.5E-8(100)[A]	CS(0.02,0.005)
10580	-	0.04	0.5	I	957	4.5E-7(87)[A]	CS(0.25,1)

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) PREVIOUSLY REPORTED - 1988. c) NALOXONE AD50 VS. NIH 10551 ED80 IN TF = 3.7. d) NALOXONE AD50 VS. NIH 10570 ED80 IN TF = 0.02. NALOXONE AD50 BEFORE NIH 10570 INTRODUCTION= 0.035. e) PREVIOUSLY REPORTED - 1987.

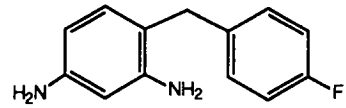
f) μ SELECTIVE ANTAGONIST (REPORTED INACTIVE IN 1988). PRIOR WORK (IN VIVO, 1987) INDICATED THAT THE COMPOUND HAD ONLY AGONIST ACTIVITY.

'ERROR IN STRUCTURE IN MCV COMPILATION IN 1988.

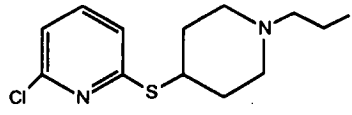
TABLE 8. MISCELLANEOUS[a]



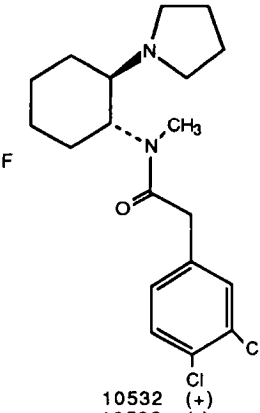
9512 (BACLOFEN)



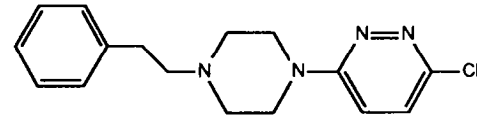
10446



10447: R=*n*-PROPYL
10448: R=ISOPROPYL



10532 (+)
10533 (-)

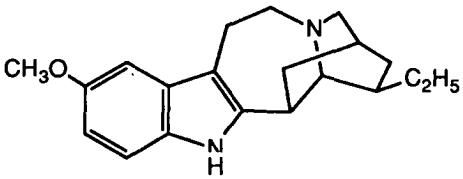


10496

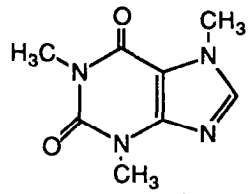
NIH#	MOUSE ED50/AD50 IN VITRO HP	PPQ	TF	TFA	RBH	VD	MONKEY SDS	NW
9512	2.1[b]	1.2[b]	I[b]	I[b,c]	-	-	NS[b]	NP[d](0.5,8)
10446	I	0.96	I	I	-	-	NS(4,12)	-
10447	I	2.6	I	I	NE	3E-5(34)[NA][e]	NS (0.6,3)[f]	NP(1,4)
10448	I	I	I	I	NE	3E-5(43)[NA][e]	NS(0.06,0.3)[f]	NP(1.2,4.8)
10496	I[g]	3.1[g]	I[g]	I[g]	1800[g]	NE[g]	NS(2.5,10)[g,h]	-
10532	I[i]	6.5[i,j]	I[i]	I[i]	>12μM[i]	1E-6(60)[A][i,k]	-	-
10533	8.9[i]	0.2[i,l]	2.5[i,l]	I[i]	4140[i]	2E-8(94)[A][i,k]	NS(0.25,3)[i,m]	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) PREVIOUSLY REPORTED - 1979. c) PREVIOUSLY REPORTED AS ACTIVE. d) INCREASED INCIDENCE OF CERTAIN WITHDRAWAL SIGNS. e) OPIOID ANTAGONIST. f) MAY EXACERBATE WITHDRAWAL. g) PREVIOUSLY REPORTED - 1987. h) OTHER - SI-HIGH; DD-CS. i) PREVIOUSLY REPORTED - 1988. j) NALOXONE AD50 VS. ED80 OF NIH 10532 = 67% AT 40. k) SIGNIFICANT κ-RECEPTOR ACTIVITY. I) NALOXONE AD50 VS. ED80 OF NIH 10533 IN PPQ AND TF = 0.7 AND 1.0, RESPECTIVELY. m) HIGH DOSE CAUSED DISORIENTATION, STUPOR.

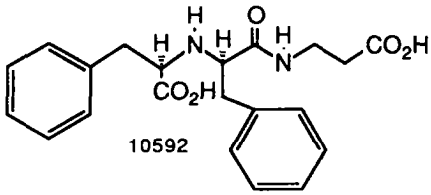
TABLE 9. MISCELLANEOUS (CONTINUED)[a]



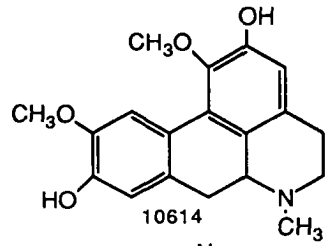
10567 (IBOGAINE)



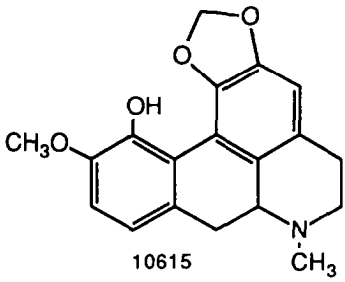
10613



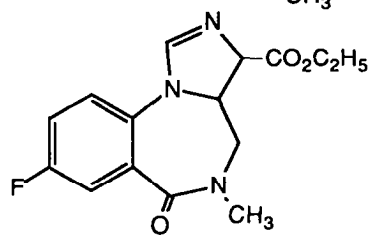
10592



10614



10615



10616

NIH#	HP	MOUSE ED50/AD50 IN VITRO PPO	TF	TFA	RBH	VD	MONKEY SDS	OTHER	PR
10567	I	9.7	I	I	6μM	2.3E-5(100)[NA]	PS(2,8)	-	-
10592	-	I	I	I	>10μM	8E-9(30)[A]	NE(3,12)	SI[b]	-
10613	-	I	I	-	-	-	-	-	-
10614	-	15.6	I	I	-	-	-	-	-
10615	-	4.5	I	I	-	-	-	-	-
10616	-	I	I	I	-	-	-	-	-

a) SEE TEXT FOR EXPLANATION OF COLUMN HEADINGS AND ABBREVIATIONS. b) MARKED VARIABILITY IN RESPONSE RATES BETWEEN ANIMALS.

ABBREVIATIONS USED IN TABLES 1 - 9.

1) MOUSE ED50 OR AD50: Antinociceptive Assays (sc injection)

Confidence limits for the ED50 and AD50 are listed in the MCV and UM reports (Aceto et al. 1990; Woods et al. 1990)].
HP = hot plate; **N** = Nilsen; **PPO** = phenylquinone; **TF** = tail flick; **TFA** = tail flick antagonism vs. morphine. These assays are performed at MCV, except for the HP and N (carried out at NIDDK, NIH). **I** = inactive, without a reasonable dose-response relationship. or insufficiently active for statistical analysis.

2) In Vitro Determinations (Data from UM)

A) **RBH** = binding affinity, in the presence of 150mM NaCl, to rat or monkey cerebrum membrane preparations, in nM (parenthesized number, noted in previous reports, is the +sodium/-sodium [+Na/-Na] ratio). EC50 was determined by displacement of 0.5 nM [^{3}H]etorphine. The EC50 of morphine from rat preparations is, for comparison = 23.6 (1.69). **NE** = no effect.
The efficacy of a few drugs on specific opioid receptors has been examined, using different radioligands (see Woods et al. 1990).

NOTE: The present EC50 data cannot be directly compared with those from some previous reports (Jacobson 1984. and preceding years) in which -Na values were quoted. However, the former numbers can be recalculated for comparison with those which arc currently utilized through the use of the +Na/-Na ratio.

B) **VD** = electrically stimulated mouse vas deferens EC50 values. rounded to one significant figure. Agonist activity is stated using "E" followed by a negative number: E = 10^{-x} M, where x = the negative number, thus: 1E-3 = 0.001 M (1 mM), 1E-6 = 1 uM, and 1E-9 = 1 nM (parenthesized numbers are maximum percent inhibition at EC50).
NE = no effect on inhibition of twitch; **SE** = slight effect on twitch; [bracketed letters: **A** = antagonized by 10^{-7} M naltrexone; **NA** = not antagonized by naltrexone; **SA** = slight antagonism by naltrexone].

Compounds which suppress the twitch and are not antagonized by naltrexone or UM 979 [NIH 8859, (-)-5,9α-dimethyl-2-(3-furylmethyl) -2'-hydroxy-6,7-benzomorphan] are said to be non-opioid agonists (e.g. clonidine can suppress the twitch, but is not antagonized by naltrexone. It is a non-opioid agonist). (The effect of UM 979 is not noted in this report, but see the UM report (Woods et al. 1990) for these data). Compounds which bind with reasonable affinity in the rat brain homogenate assay, suppress the twitch in the VD, but are not blocked by narcotic antagonists may have narcotic antagonist properties. This is experimentally determinable by observing their antagonism to morphine's suppression of the twitch in the VD preparation (for these data see Woods et al. 1990).

3) Monkey Colony Data (from MCV; prior to 1988 from MCV or UM.

A) **SDS** = single dose suppression
NS = no suppression; **CS** = complete suppression; **PS** = partial suppression. (Parenthesized numbers = dose range studied, in mg/kg; if CS, then dose at which CS was observed is noted in the parentheses). Potency comparison with morphine [M] may be stated, in brackets.

B) **NW or PPt-W** studies in non-withdrawn monkeys
PW = precipitated Withdrawal at dose levels, in mg/kg, indicated in parentheses &/or comparison with naloxonc [N], in brackets; **NP** = no precipitation; **SP** = slight precipitation.

4) Other Studies (OTHER):
A) **RI** = rat continuous infusion (data from MCV)
a) **SM** = substitution for morphine
NS = no substitution for morphine; **CS** = complete substitution;
PS = partial substitution].
b) **PPD** = primary physical dcpendcncc. in rats.

B) **ND** = non-deoendent monkeys
M-like = morphine-like effect.

C) **PPD** = primary physical deoendcncc (in the rhesus monkey).

D) **SA** or **SI** = self-administration or self-inicction (data from UM)
NE = no effect; **High** = codeine-like; **IN** = intermediate between saline and codeine; **SE** = slight effect.

E) **DD** = drug discrimination (data: from UM)
NE = no effect, **CS** = complete suppression.

Previous Reports

Previous work on a compound is noted by year, the year listed in the monograph title (e.g. Problems of Drug Dependence 1986). Note that the date of publication of the monograph generally occurs one year after the titled year of the monograph. The data which have been published in previous reports are shown in the tables in the appropriate -column and the year in which the original work can be found is cited in the footnotes to the tables (e.g., a 1983 previous report would indicate that the work was cited in "Problems of Drug Dependence 1983". which was published in 1984. The work can be found in the Annual Report of either Aceto et al., or Woods et al.).
NOTE: Rounded numbers arc used in the tables. For precise values, and details of the procedures, see the MCV and UM reports in these Proceedings (Aceto et al. 1990; Woods et al. 1990).

Abbreviations for structural formulae:
CPM = cyclopropylmethyl.

THE STIMULANT AND DEPRESSANT PROGRAM

Of the seven compounds which were examined this year, three were received from pharmaceutical industry at the urging of the WHO, since biological data on these three compounds were needed for discussion at the Expert Committee meeting of WHO in April, 1989. These compounds, ctizolam, brotizolam, and quazepam (CPDD 0024, 0025, and 0026, respectively) are benzodiazepine-like in structure (figure 1) and were examined as depressants.

Brotizolam. CPDD 0025, was examined by self-injection (SI) procedures at UM and appeared to have only a slight reinforcing effect. However, administration of CPDD 0025 resulted in drug appropriate responding in a drug discrimination assay, at the University of Chicago, and tolerance appeared to result from the discriminative stimulus and rate-altering effects of the compound.

Etizolam and quazcpam were also examined in drug discrimination. Administration of etizolam (CPDD 0024) resulted in drug appropriate responding IO all subjects tested (up to 3 mg/kg, generalization to a pentobarbital stimulus). Quazepam (CPDD 0026) also produced discriminative stimulus effects similar IO those 'of pentobarbital.

All three of these compounds would be predicted. from drug discrimination assays, to have pcntobarbital-like subjective effects in humans.

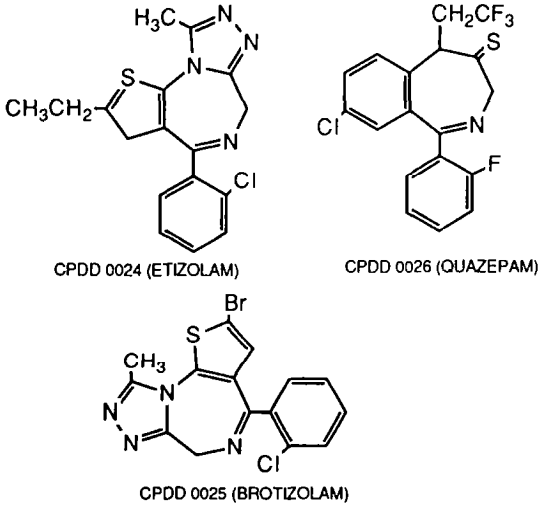


FIGURE 1. Structures of ctizolam, brotizolam and quazepam

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ACKNOWLEDGEMENT

The author thanks Mariena V. Mattson, NIDDK, LN, for the skillful performance of the hot plate assay.

AUTHOR

A. E. Jacobson, Ph.D., Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse (1989)

M. D. Aceto, E. R. Bowman, L. S. Harris and E. L. May

The identities of the test compounds were unknown to us when they were originally submitted except for NIH 10616 (Flumazenil). Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIADDK, NIH, supplied all the compounds except caffeine. This study was done under the auspices of the Committee on Problems of Drug Dependence, Inc.

For the most part. the procedures described by Seevers and his colleagues (1936. 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3.0 mg/kg s.c. of morphine sulfate every 6 hr for at least 90 days before being used. This dose regimen was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single-dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2 1/2 hr after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hr after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test drug were noted In both tests, a vehicle control and an appropriate positive control (naloxone hydrochloride, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 2 or 3 different treatments (doses) of a test compound were randomly allocated to the 4 or 5 monkeys of a group. Usually, 3 or 4 groups per compound were used. All drugs were given subcutaneously (1 ml/kg) and the vehicle was water except where indicated. The observer was "blind" with regard to the treatment given. A minimal 2-week washout and recuperation period between tests was allowed. In the primary physical dependence (PPD) tests, the animals of a group received the drug every 4-6 hr for 30-50 days. They were placed in abrupt withdrawal and challenged with naloxone periodically, then observed for signs of physical dependence. All potency estimates are rough approximations only.

The rat-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Semi-restrained, male, Sprague-Dawley rats were medicated with a drug by continuous infusion through indwelling intraperitoneal cannulas for 6 days. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a

flow-through swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 hr.

In the substitution for morphine (SM) test, the animals first received morphine (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr from days 3-6). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of water. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 1/2 hr at 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

In the primary physical dependence (PPD) study, the rats received test compound for 6 days and then were placed in abrupt withdrawal and observed as above. Occasionally, a drug was given with morphine.

Three mouse tests were used in our laboratory to provide a preliminary estimate of the potency and profile of activity of each test compound. The tests were the tail-flick agonist (TF) and the morphine antagonist (TF vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in Table 1. In addition, Dr. Jacobson occasionally provided us with estimated starting doses. These doses were based on results obtained from the mouse-hot plate (HP) (Eddy and Leimbach. 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978) and Nilsen (N) (Perrine et al., 1972) tests from his laboratory. Reference data for these tests are shown in Table 2.

Table 1

Comparative Data-ED50, mg/kg S.C. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick Test	Tail-Flick Antagonist Test	Phenylquinone Test
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)
Cyclazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01(0.005-0.03)
Nalorphine·HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)
Naloxone·HCl	None at 10.0	0.04 (0.01-0.09)	No Activity
Naltrexone·HCI	None at 10.0	0.007 (.002-0.02)	No Activity
Morphine Sulfate	5.8(5.7-5.9)	-----	0.23(0.20-0.25)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time.

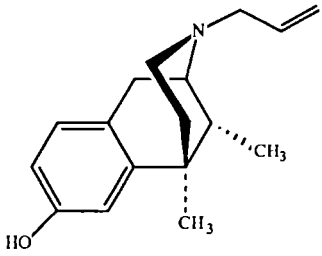
Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate and Nilsen Assays

	Hot Plate s.c./p.o.	Nilsen s.c./p.o.
Morphine Sulfate	0.98 (0.83-1.1) 6.3 (4.7-8.3)	1.3 (1.0-1.7) 8.3 (6.0-11.4)
Codeine Phosphate	6.8 (4.5-10.2) 13.5 (9.7-18.7)	7.4 (4.9-11.0) 14.7 (9.2-23.3)
Levorphanol Tarn-ate	0.2 (0.1-0.3) -	0.2 (0.16-0.3) 2.5 (1.7-3.7)
Meperidine·HCl	5.3 (4.0-7.1) -	- -
(-)-Metazocine·HBr	0.6 (0.5-0.9) 10.6 (8.0-14.1)	0.5 (0.3-0.7) 26.0 (21.0-33.0)
Dihydromorphinone·HCl	0.19 (0.15-0.25) 0.9 (0.7-1.2)	0.2 (0.15-0.3) 1.8 (1.5-2.1)
Nalorphine·HCl	9.9 (5.7-2.1) -	23.0 (16.2-32.7)
Cyclazocine	1.5 (1.1-2.1)	0.1(0.07-0.16)
Pentazocine	9.3 (6.7-12.8) -	6.5 (4.4-8.8) -
Chlorpromazine·HCl	1.1 (0.9-1.5) -	- -

No dose response for naloxone and naltrexone.
Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive on the hot plate test.

NIH 7912 (±)-N-allylnormetazocine, (±)-SKF 10,047, (±)-NANM

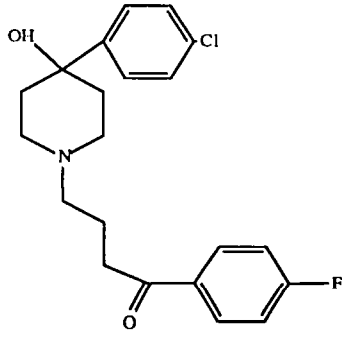


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 1.9 (0.7 - 6.0)
3) PPQ - 10.4 (5.3 - 20.3)
4) HP - Inactive to 50.0
5) N - Inactive to 50.0

A. Special Study: AD50 of NIH 7912 vs ED80 of morphine in PPQ Test = 3.3 (1.9 - 9.5)
B. Special Study: AD50 of naloxone vs ED80 of NM 7912 in PPQ Test = 3.4 (1.9 - 9.8)
C. Special Study: AD50 of yohimbine vs ED80 of NIH 7912 in PPQ Test = 0.9 (0.3 - 2.2)

NlH 8032, Haloperidol



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 14.6 (10.9 - 19.5)[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.011 (0.002 - 0.048)

Rodent data reported previously.

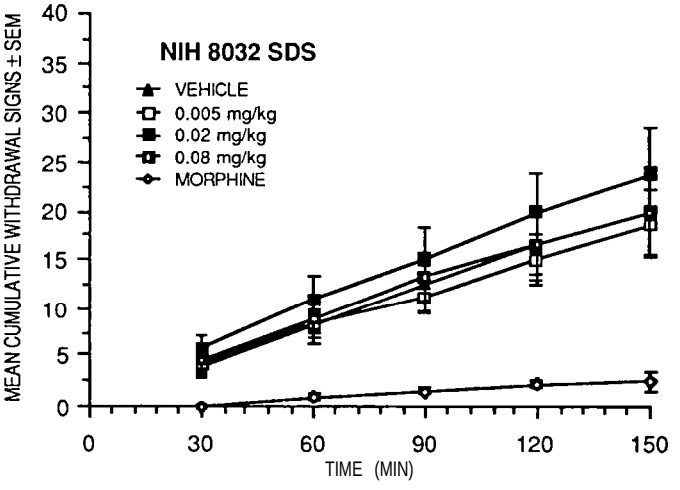
[a]Very erratic results. Naloxone produced 65% antagonism vs the ED60 of haloperidol.

MONKEY DATA
(SDS)

This study was initiated to provide comparative data for the evaluation of certain N-butyrophenone-prodine compounds (NIH 10494 and NIH 10495). which were found to have opioid/neuroleptic properties. This study was also prompted by the observation, in a previous study. that a normally docile male rhesus monkey, when medicated with haloperidol, became unusually aggressive towards his handler. The doses selected in this study were not in the range where severe neuroleptic signs were observed.

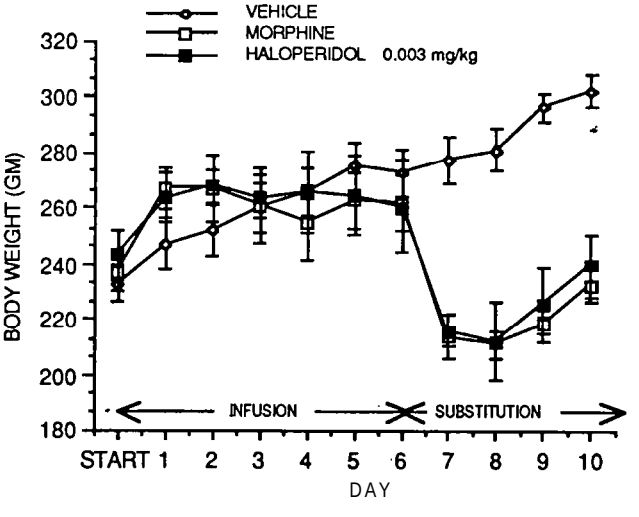
As can be seen in the fig., haloperidol did not substitute for morphine. At the highest dose, more retching and tremors were observed than with the vehicle

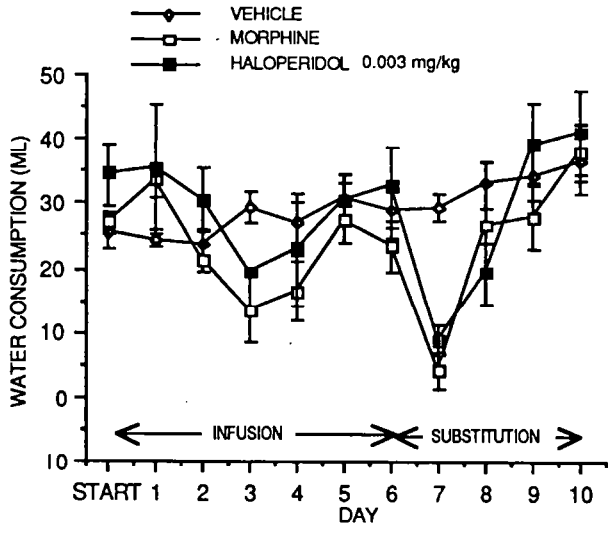
controls. In addition, some of these animals appeared slower and subdued. At the two lower doses, more restlessness and retching were noticed than in the controls. In this dose range, none of the animals exhibited aggressive behavior.



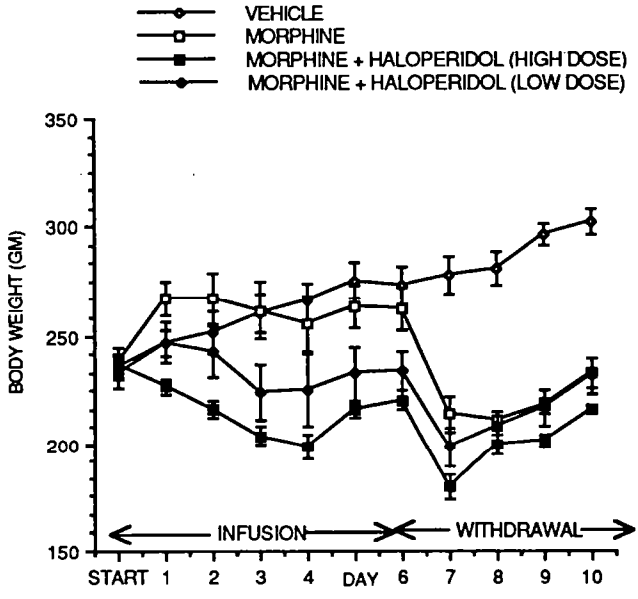
RAT INFUSION

A) Substitution for morphine (R-SM) As can be seen from the data on body weight and water consumption (figs.) and overt signs (table), haloperidol did not substitute for morphine at a dose of 0.003 mg/kg/day. At this dose, no overt neuroleptic signs were detected.





B) Special Morphine Plus Haloperidol Primary Physical Dependence Study (SR-PPD) When morphine plus haloperidol, at 2 dose levels, and morphine were given and then abruptly withdrawn after 6 days, the withdrawal syndromes were qualitatively similar to each other. Quantitatively, at 24 hrs, the animals receiving haloperidol and morphine showed more weight loss and overt withdrawal signs than those receiving morphine only or vehicle. This was in spite of the fact that the animals receiving the combination of drugs showed severe disturbances such as weight loss, changes in water consumption and neuroleptic behavioral signs during the administration of drugs.


VEHICLE
MORPHINE
MORPHINE + HALOPERIDOL (HIGH DOSE)
MORPHINE + HALOPERIDOL (LOW DOSE)
BODY WEIGHT (GM)
350
300
250
200
150
INFUSION
WITHDRAWAL
START 1 2 3 4 DAY 6 7 8 9 10

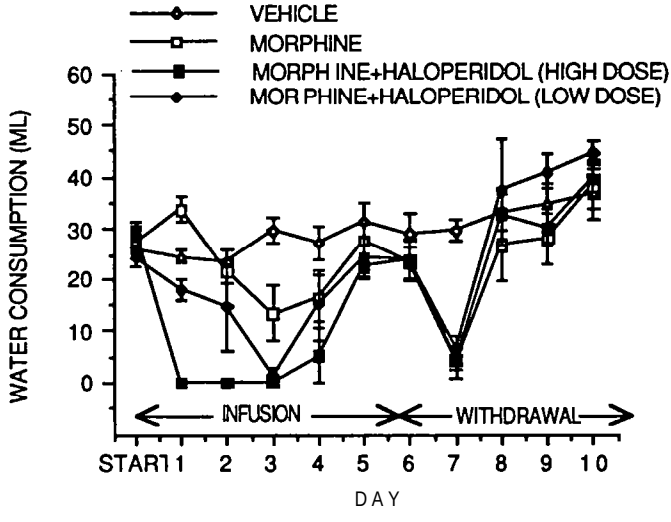

VEHICLE
MORPHINE
MORPH INE+HALOPERIDOL (HIGH DOSE)
MOR PHINE+HALOPERIDOL (LOW DOSE)
WATER CONSUMPTION (ML)
60
50
40
30
20
10
0
INFUSION
WITHDRAWAL
START 1 2 3 4 5 6 7 8 9 10
DAY

Table. Evaluation of overt withdrawal signs observed in the infusion studies.

Rat Infusion Studies: Haloperidol substitution for morphine and haloperidol and morphine simultaneously.

	Overt Withdrawal Signs				
	24hr	48hr	72hr	90hr	120hr
Vehicle Infusion[b] N=4	0.0	0.6	0.3±3	1.5±1.5	0.0
Morphine Infusion[d] Vehicle Substitution N=3	9.0±3.5[c]	9.0±2.3[c]	6.3±3.9	2.0±1.2	1.0±0.7
Morphine Infusiond Halopetidol Substitution[e] N=3	7.7±3.2[c]	11.0±5.9[c]	3.7±2.2	1.3±0.9	1.7±1.7
Morphine plus Haloperidol Infusion[d,f], Vehicle Substitution (high-dose regimen) N=3	12.7±4.3[c]	8.0±13.8[c]	4.3±2.4[c]	2.0±1.5	1.0±0.6
Morphine plus Haloperidol[d,g] Infusion, Vehicle Substitution (low-dose regimen) N=3	16.0±4.5[c]	7.3±1.6[c]	5.3±1.6[c]	2.3±0.5	0.3±0.3

[a]Mean number of opioid-like withdrawal signs ± S.E.M. noted in a 1/2 hr observation period at specified intervals. Signs are hypersensitivity, squealing, hypersensitivity, aggression, wet-dog shakes, rubbing and chewing.

[b]veticle volume was 8 ml/24 hr days 1-10.

[c]statistically significant differences (p = 0.05 or less) between Vehicle only and treated group. One-tailed test (Mann-Whitney test).

[d]Morphine SO_4 infusion - 50 mg/kg day 1, 100 mg/kg day 2, and 200 mg/kg days 3-6.

[e]Haloperidol infusion 3.0 mg/kg day 1, 1.5 mg/kg day 2, 0.5 mg/kg day 3, 0.01 mg/kg days 3-6 (high dose)

[f]Haloperidol infusion - 0.03 mg/kg on days 7 and 8, Vehicle on days 9 and 10

[g]Haloperidol infusion 0.5 mg/kg day 1, 0.15 mg/kg day 2, 0.003 mg/kg days 3-6 (low dose).

268-365 0 - 90 - 20 : QL 3

NIH 8773- (-)-N-allylnormetazocine, (-)-SKF 10,047, (-)-NANM

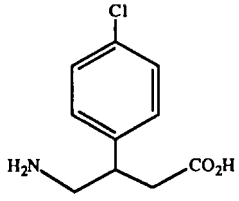
	MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)
See NIH 7912	1) TF - Inactive at 1.0, 10.0 and 30.0 2) TF vs. M. - 0.2 (0.1 - 0.6) 7.1 3) PPQ - 1.3 (0.4 - 5.0) 10.3[a,b,c] 4) H P - 5) N - Inactive to 50.0

A. Special Study: AD_{50} of naloxone vs ED_{80} of NIH 8773 in PPQ Test = 0.13 (0.05 - 0.3)
B. Special Study: AD_{50} of NIH 8773 vs ED_{80} of morphine in PPQ Test = 1.2 (0.6 - 2.3)
C. Special Study: AD_{50} of yohimbine vs ED_{80} of NIH 8773 in PPQ Test = 0.2 (0.1 - 0.3)

NIH 8775 (+)-N-allylnormetazocine, (+)-SKF 10,047, (+)-NANM

	MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)
See NIH 7912	1) TF - Inactive at 1.0, 10.0 and 30.0 2) TF vs M. - 13.2 (6.6 - 26.2) 30.0 3) Inactive to 40.0 4) HP - Inactive to 50.0 5) N - Inactive to 20.0

NIH 9512 Baclofen, Lioresal



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg or % change)

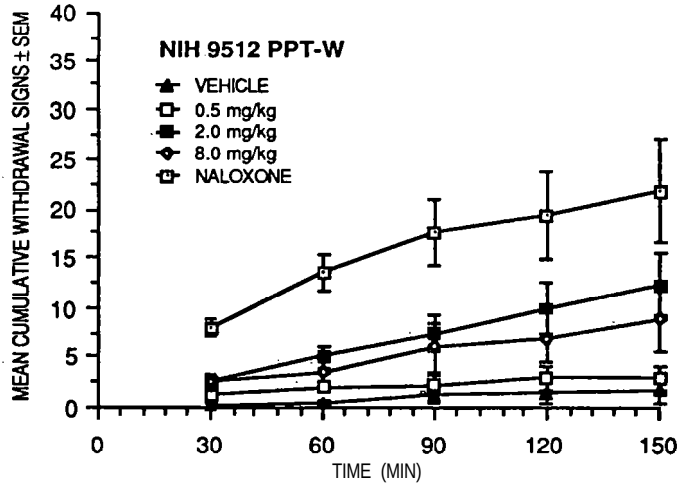
1) TF - 0% at 1.0, 45% at 3.0, 64% at 10.0 and 3% at 30.0
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 1.2 (0.4 - 3.1)
4) HP - 2.1 (1.5 - 2.7)
5) N - Inactive to 20.0

[a]Previously reported as very active [AD50 - 0.06 (0.02 -0.17)] in NIDA Monograph 27, 1979.

MONKEY DATA
(PPt-W)

NIH 9512 was studied in the single-dose suppression (SDS) test in monkeys (NIDA Monograph 27, 1979). It did not substitute for morphine. In order to fully investigate possible antagonist properties, a precipitated withdrawal test was conducted.

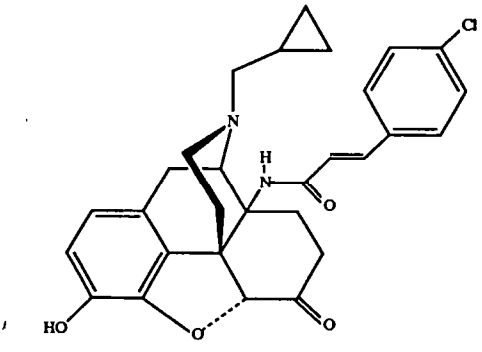
In non-withdrawn, morphine-dependent monkeys, this compound increased the incidence of certain withdrawal signs designated fighting, avoids contact, vocalizing and in one monkey, at the high dose, retching, vomiting and coughing. In addition, at 2.0 mg/kg. 2 monkeys vocalized when their abdomens were palpated and had rigid abdominal muscles. Thus, although the compound increased the incidence of certain withdrawal signs, it did not precipitate a full withdrawal syndrome. The vehicle was H_3P0_4 and H_20.



Conclusion

This compound does not show antagonist activity either in the mouse antinociception vs morphine assay or morphine-dependent monkeys.

NIH 10443 14ß-(*p*-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethyl-normorphinone mesylate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

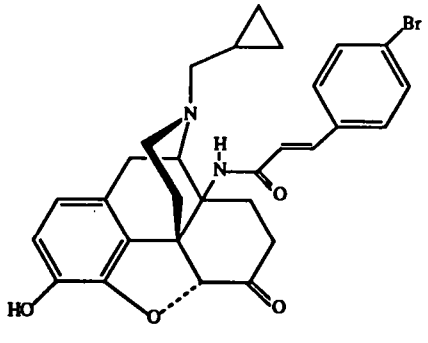
1) TF- Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - 0.12 (0.07 - 0.23)[a]
3) PPQ - 23% at 3.0, 34% at 10.0, 69% at 30.0 and 54% at 10.0[a]
4) HP - Inactive to 20.0

[a]Reported previously in NIDA Monograph 81, 1987

Special Duration Study: Morphine antagonism of NIH 10443 ED_{200}

Pretreatment Time (hr)	% Antagonism
24	76
48	19
72	18

NIH 10445 14ß-(*p*-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropyl-methylnormorphinone mesylate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

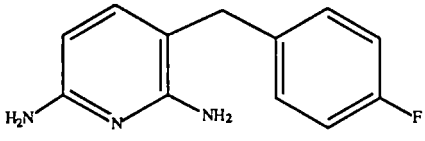
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - 0.8 (0.6 - 1.0)[a]
3) PPQ - 7.1 (3.1 - 16.4)[a]
4) HP-Inaetive to 20.0[a]

[a]Reported previously in NIDA Monograph 81, 1987

Special Duration Study: Morphine antagonism of NIH 10445 ED_{200}

Pretreatment Time (hr)	% Antagonism
24	80
48	57
72	36
96	29
120	1

NIH 10446 2,6-Diamino3-*p*-fluorobenzylpyridine

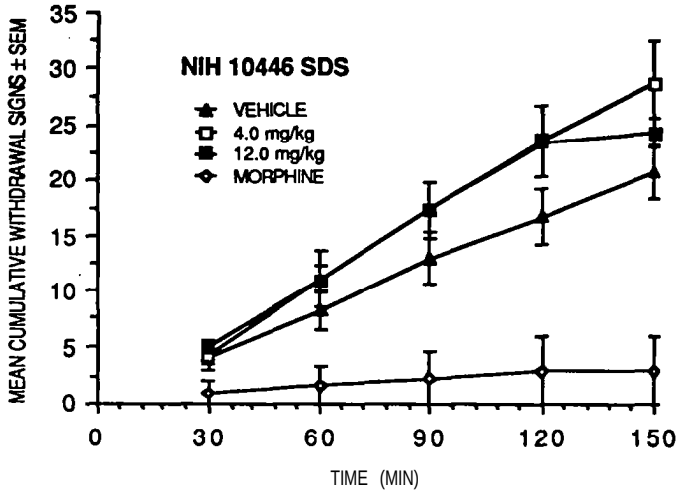


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

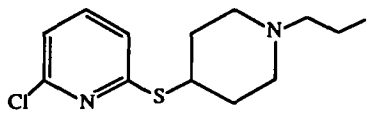
1) TF - 3% at 0.1, 20% at 1.0, 6% at 1.00 and 0% at 30.0
2) TF vs. M. - 22% at 1.0, 0% at 10.0 and 0% at 3.0
3) PPQ - 0.96 (0.31 - 2.98)
4) HP - Inactive at 5.0 and 20.0

MONKEY DATA
(SDS)

In the dose range of 4.0 - 12.0 mg/kg, NIH 10446 did not substitute for morphine. The drug may have exacerbated withdrawal (see fig.). One animal receiving 4.0 mg/kg showed myoclonic jerks.



NIH 10447, MCV 4517 2-Chloro-6-(4-N-*n*-propylpiperidino)thiopyridine hydrochloride



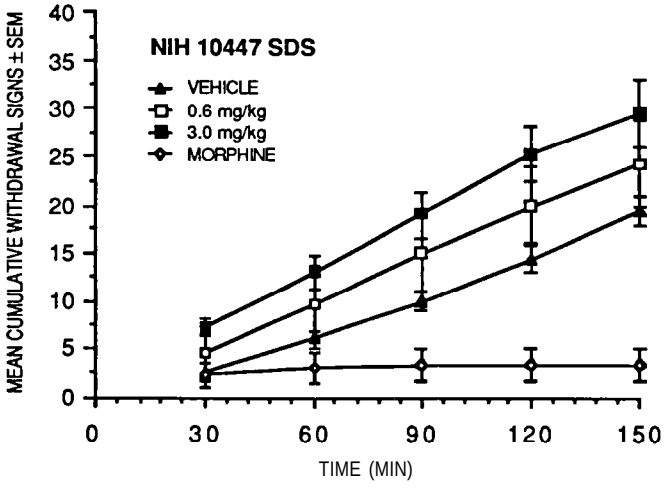
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 27% at 1.0, 47% at 10.0 and 16% at 30.0
3) PPQ - 2.6 (0.5 - 12.7)
4) HP - Inactive to 20.0

MONKEY DATA

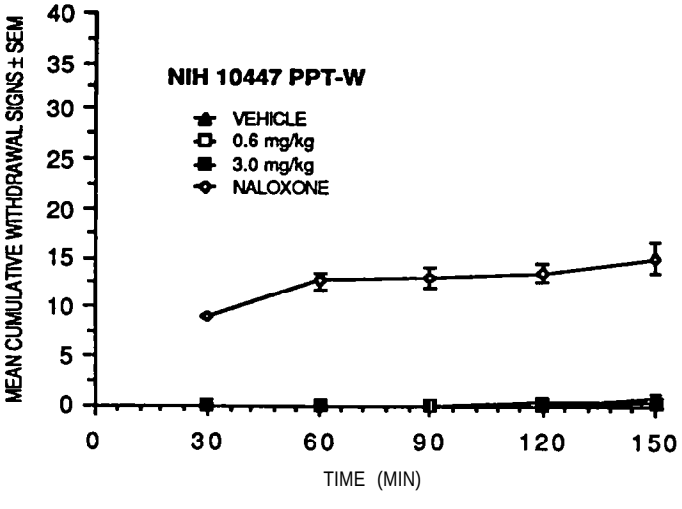
A. (SDS)

NIH 10447 does not substitute for morphine. The drug seemed to exacerbate withdrawal (Fig. 2); however, this may be a reflection of the fact that the vehicle controls showed an unusually weak abrupt-withdrawal syndrome.

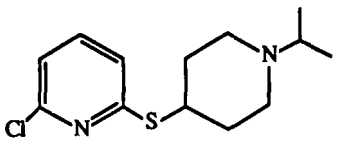


B. (PPt-W)

As shown in the figure, NIH 10447 was inactive in precipitating withdrawal in morphine-addicted monkeys. Two animals receiving the highest dose were very drowsy and moved abut slowly one hour after drug was given. Another, at the highest dose was not as aggressive as usual.



NIH 10448, MCV 4518 2-Chloro-6-(4-N-isopropylpiperidino)thiopyridine oxalate

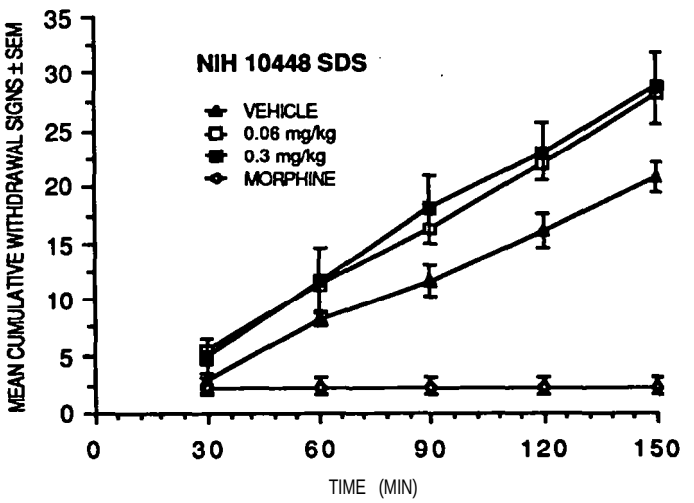


MOUSE DATA-ED OR AD50 (95% C.L.) (mg\kg or % change)

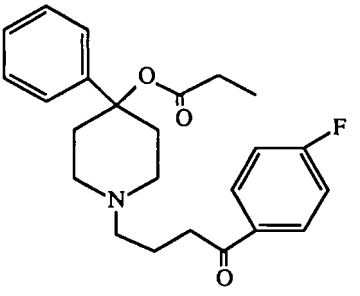
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 37% at 0.1, 40% at 1.0, 54% at 10.0 and 84% at 30.0
4) HP - Inactive to 20.0

MONKEY DATA
(SDS)

This compound does not substitute for morphine (see fig.). The drug may have exacerbated withdrawal although the effect does not seem to be dose related.



NIH 10495, MCV 4560 N-3-(*p*-Fluorobenzoyl)propyl-4-phenyl-4-propionyloxy piperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

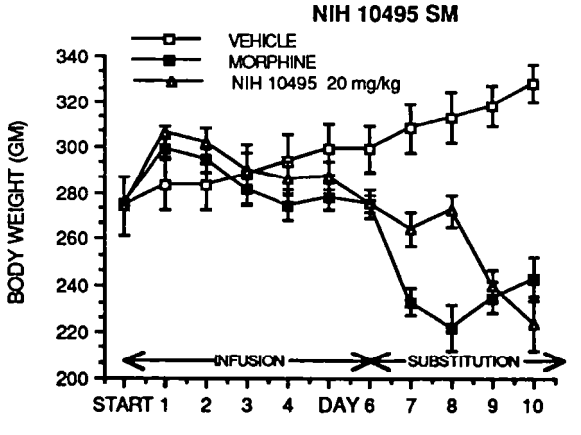
1) TF- 0.3 (0.1 - 1.1)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.07 (0.02 - 0.18)
4) HP - 0.32 (0.25 - 0.42)

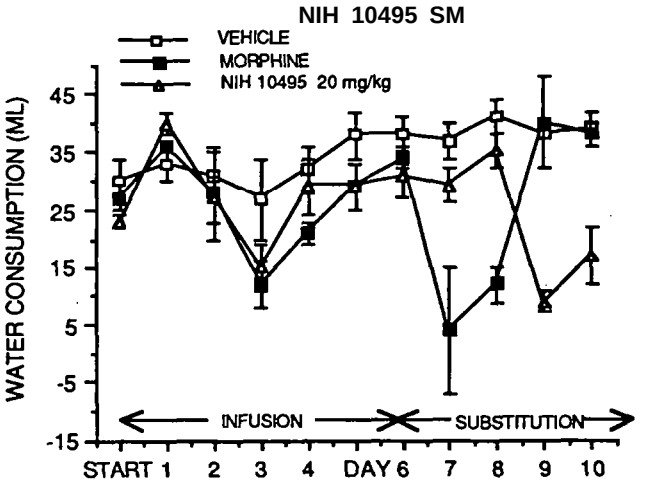
Rodent and monkey data reported in NIDA Monograph 90, 1988.

RAT INFUSION

A. (SDS)

As shown in the figs. (body weight loss and water consumption), NIH 10495 partly substituted for morphine at 20.0 mg/kg. Regarding behavioral signs (see table), the drug nearly substituted for morphine. It is possible that the drug has a delayed onset of action since body weight loss, water consumption and behavioral withdrawal were less on days 8 than day 7. Withdrawal signs reemerged on days 9 and 10 after the drug was withdrawn and vehicle substituted.



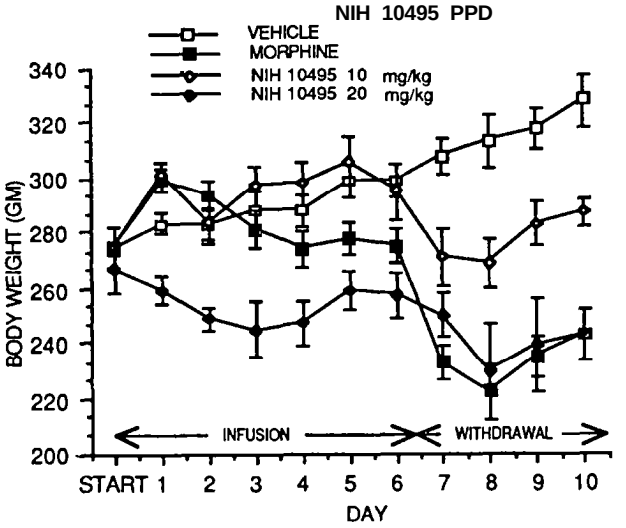


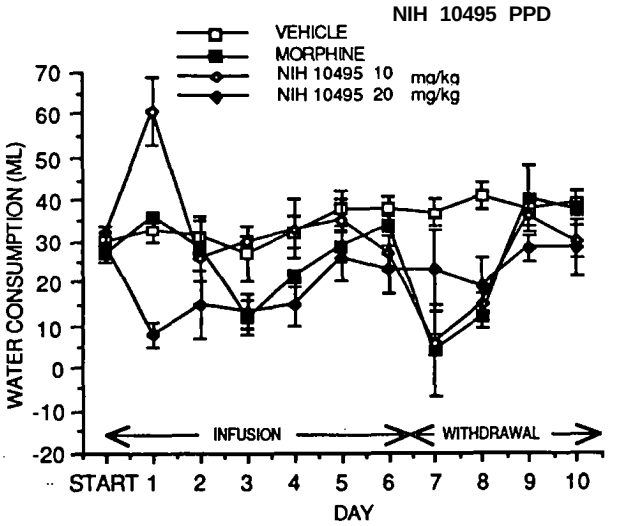
Rat Infusion - Cont'd

B. (PPD)

NIH 10495 produced a dose-related fall in body weight and drop in H_20 consumption (see figs.) and dose-related increases in withdrawal signs (see table) when withdrawn after 6 days of continuous infusion. During the first day of infusion, the drug produced unusual changes in the H_20 consumption, the low dose increased and the high dose decreased consumption dramatically. Whether or not this is a spurious happening is uncertain.

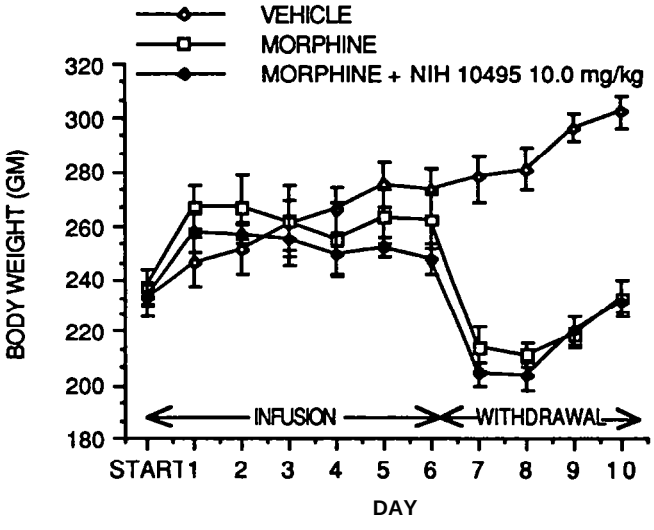
In any case, a physical dependence syndrome, similar to morphine's, develops with this agent


NIH 10495 PPD
VEHICLE
MORPHINE
NIH 10495 10 mg/kg
NIH 10495 20 mg/kg
BODY WEIGHT (GM)
340
320
300
280
260
240
220
200
INFUSION
WITHDRAWAL
START 1 2 3 4 5 6 7 8 9 10
DAY


NIH 10495 PPD
VEHICLE
MORPHINE
NIH 10495 10 mg/kg
NIH 10495 20 mg/kg
WATER CONSUMPTION (ML)
70
60
50
40
30
20
10
0
-10
-20
INFUSION
WITHDRAWAL
START 1 2 3 4 5 6 7 8 9 10
DAY

C. Special Study: Morphine + NIH 10495 R-PPD

The withdrawal syndrome resulting from the abrupt withdrawal of a solution containing morphine and NIH 10495 was qualitatively and quantitatively similar to that produced by the morphine controls (see figs. and table).



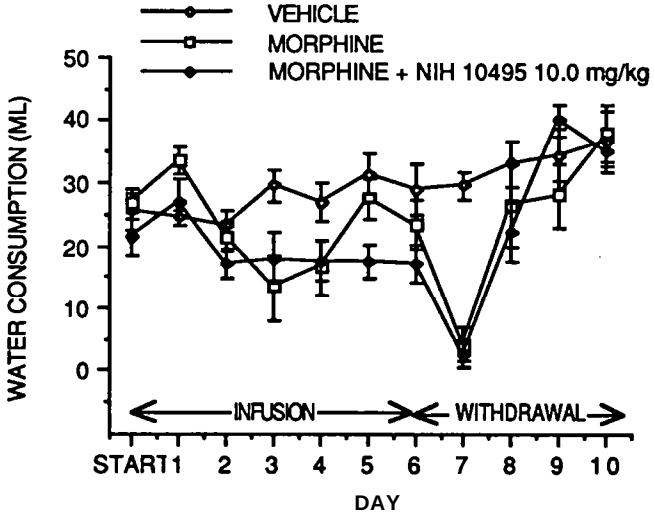


Table: Primary Physical Dependence (PPD) and Substitution for Morphine Studies (SM) with NIH 10495 in Continuously-Infused Rats

Treatment	Hr in Withdrawal			
	24	48	72	96
	Mean Number of Withdrawal Signs [a,b]			
1. Vehicle Controls[c]	0.5	1.5	0	1.3
2. Morphine Controls[d]	14.2[b]	20.0[b]	9.0[b]	2.0
3. NIH 10495-PPD[e] (high dose)	9.3[b]	9.3[b]	8.0	3.7
4. NIH 10495-PPD[f] (low dose)	12.5	6.5	5.3	2.3
5. NIH 10495-SDS[g] (high dose)	3.8	0.8	12.8[b]	8.0[b]

[a]Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing;

[b]One-tailedtest Mann-Whitney U test, $p < 0.05$, probability value vs. water controls;

[c]8 ml/24 hr. N=4;

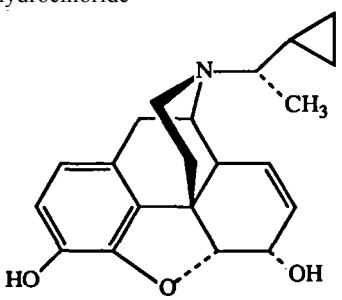
[d]Dose regimen of morphine SO_4, 50 mg/kg on day 1. 100 mg/kg on day 2, 200 mg/kg on days 3-6. N=5;

[e]Dose regimen of NIH 10495.20 mg/kg/day on days 1-6; then H_20 as above during withdrawal. N=3;

[f]Dose regimen of NIH 10495 10.0 mg/kg on days 1-6; then, H_20 as above during withdrawal. N=4;

[g]Morphine SO_4 Infusion, days 1-6 as above then, NIH 10495 on days 7 and 8, 20 mg/kg, and H_20 as above on days 9 and 10. N=5 on days 7 and 8; and 4 on days 9 and 10.

NIH 10497. MCV 4558 N-[(1**R**)-l-Cyclopropyl]ethylnormorphine hydrochloride

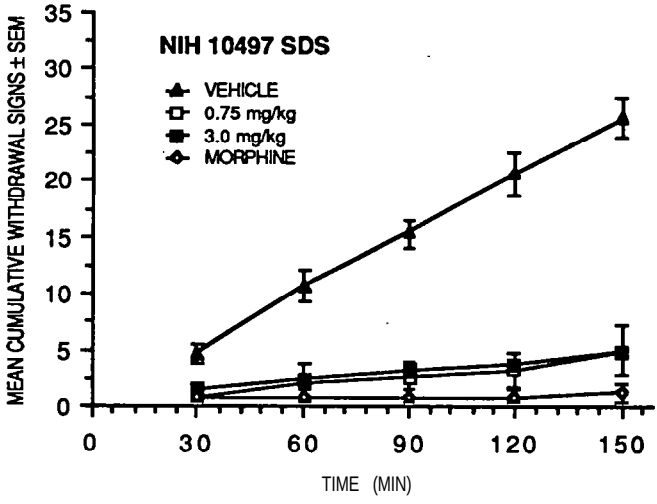


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

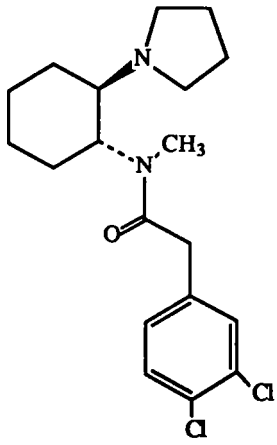
1) TP - 2.0 (0.6 - 6.6)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.03 (0.01 - 0.2)

MONKEY DATA
(SDS)

NIH 10497 substituted completely for morphine. The drug acted promptly and its duration of action was about 2 hr. (see fig.). In addition, this drug is slightly less potent than morphine. Many drug-related side effects were seen including body sag, jaw sag, slowing, staring, and salivation. The incidence of drowsiness was more than that observed in morphine-treated controls.



NIH 10532. MCV 4581 (+)-*trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl-1]benzeneacetamide *d*-tartrate, ((+)-U 50,488 *d*-tartrate)



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 0% at 1.0, 2% at 10.0 and 21% at 30.0
3) PPQ - 6.5 (2.0 - 20.9)[a]
4) HP - Inactive at 20.0

Reported previously in NIDA Monograph 90, 1988.

[a]Special Study: Naloxone vs NIH 10532 ED_{80} in PPQ test

Naloxone Dose mg/kg sc	% Antagonism
40.0	67%
20.0	64%
10.0	29%
1.0	14%

Conclusion: Very high doses of naloxone only partially antagonize the antinociceptive activity of NIH 10532 in the PPQ test.

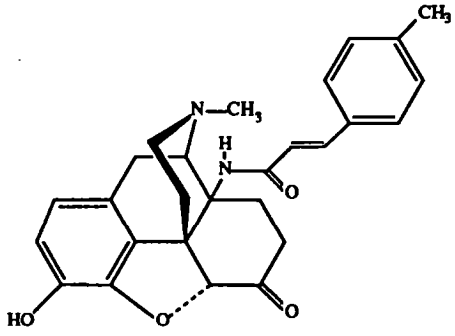
NIH 10533. MCV 4582 (-)-*trans*-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl-1] benzeneacetamide *l*-tartrate, ((-)-U 50,488 *l*-tartrate)

	MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)
See NIH 10532	1) TF - 2.5 (1.0 - 6.0)[a] 2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0 3) PPQ - 0.2 (0.08 - 0.54)[b] 4) HP - 8.9 (6.0 - 13.2) Rodent data reported previously in NIDA Monograph 90, 1988.

a. Special Study: Naloxone vs NIH 10533 ED_{80} in TF test = 0.7 (0.2 - 3.2)

b. Special Study: Naloxone vs NIH 10553 ED_{80} in PPQ test = 1.0 (0.3 - 2.9)

NIH 10544 mesylate — 14ß-(*p*-Methylcinnamoylamino)-7,8-dihydromorphinone

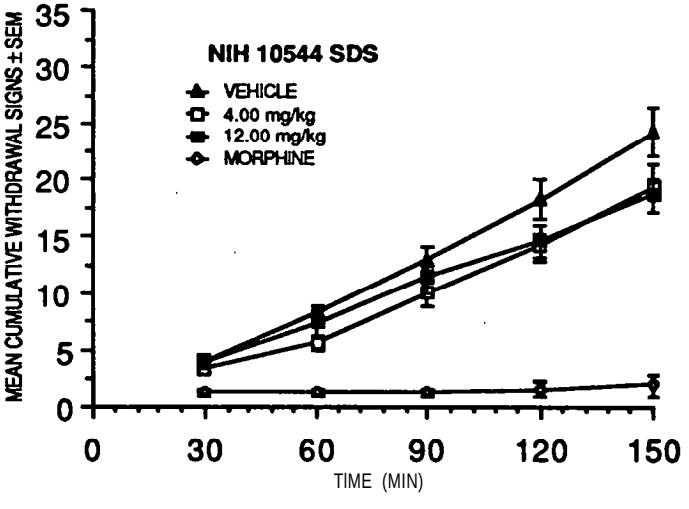


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

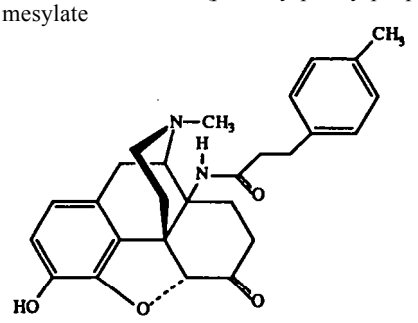
1) TF- 11% at 1.0 and 10.0 and 14% at 30.0
2) TF vs. M. - 0.6 (0.2 - 1.4)
3) PPQ - 17% at 0.1, 57% at 1.0, 57% at 3.0, 63% at 10.0, 51% at 30.0 and 69% at 60.0
4) HP - Inactive at 2.0 and 10.0

MONKEY DATA (SDS)

As shown in the accompanying graph, NIH 10544, at doses of 4 and 12 mg/kg neither substituted for morphine nor exacerbated withdrawal.



NIH 10545 14ß-(*p*Methylphenylpropionylamino)-7,8-dihydromorphinone mesylate

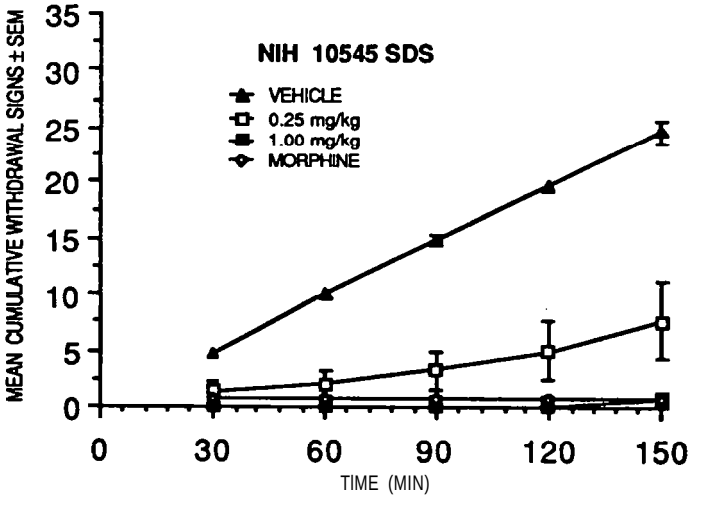


MOUSE DATA-ED OR AD50 (95% CL.) (mg/kg or % change)

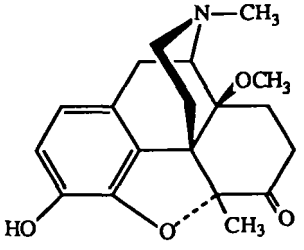
1) TF - 0.5 (0.2 - 1.5)
2) TF vs. M - 0% at 1.0 and 10.0 and 17% at 30.0
3) PPQ - 0.09 (0.03 - 0.24)
4) HP - Inactive at 2.0 and 10.0

MONKEY DATA
(SDS)

A dose-related suppression of abstinence signs in morphine-dependent and withdrawn monkeys was observed. At 1.0 mg/kg. the drug substituted completely for morphine. Onset of action was prompt offset of action was at least that of morphine (> 140 min). NIH 10454 is estimated to be 3 to 5 x more potent than morphine.



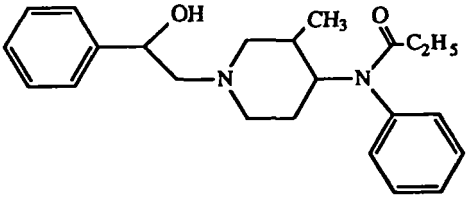
NIH 10549 14ß-Methoxy-5-methyl-7,8-dihydromorphinone hydrobromide



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.02 (0.007 - 0.05)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.003 (0.001 - 0.006)

NIH 10551 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropanamide hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.0002 (0.0001 - 0.0005)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.00013 (0.00006 - 0.0003)
4) HP - < 0.0002

Rodent data reported previously in NIDA Monograph 90, 1988.

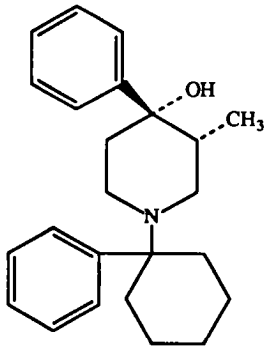
A. Special Study: Naloxone vs NIH 10551 ED_{80} in PPQ test AD50 = 3.7 (1.3 - 10.7)

B. Special Study: Naloxone vs ED80 of NIH 10551 in TF = 0.06 (0.03 - 0.1)

MONKEY DATA
(SDS)

Estimated potency in SDS test was 25,000 x morphine. SDS results reported previously in NIDA Monograph 90, 1988.

NIH 10553 (+)-4-Hydroxy-3-methy1-4-phenyl-1-(1-phenylcyclohexyl)-piperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 8.7 (4.1 - 18.3)[a]
2) TF vs. M. - Inactive at 1.0. 10.0 and 30.0[a]
3) PPQ - 0.7 (0.3 - 2.1)[a,b]
4) HP - 16% at 20.0

Vehicle-5% Tween 80 in H_20

[a]Rodent data reported previously in NIDA Monograph 90, 1988.

[b]Special Study: Naloxone vs NIH 10553 ED_{80} in PPQ test. AD_{50} = 0.06 (0.03 - 0.12)

NIH 10554 (-)-4-Hydroxy-3-methyl-4-phenyl-1-(1-phenylcyclohexyl)-piperline hydrochloride

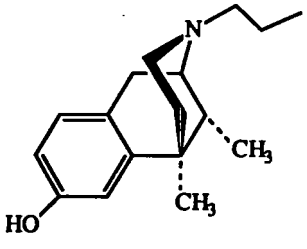
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

See NIH 10553

1) TF - 0% at 1.0, 14% at 10.0 and 34% at 30.0[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.5 (0.8 - 7.3)[a]
4) HP - Inactive to 20 mg/kg

[a]Vehicle 4% Tween 80 in H_20

NIH 10556 (+)-5,9α-Dimethyl-2'-hydroxy-2-propyl-6,7-benzomorphan hydrochloride



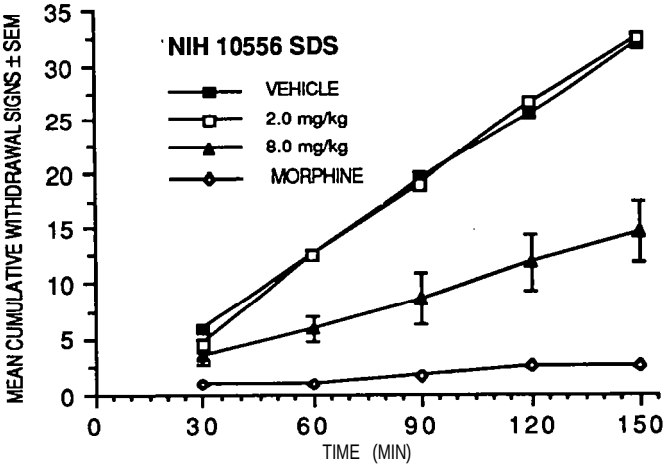
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 3.0, 10.0 and 30.0[a]
2) TF vs. M. - 0% at 1.0. 6% at 10.0 and 20% at 30.0
3) PPQ - Inactive at 1.0. 10.0 and 30.0
4) HP - Inactive to 20 mg/kg

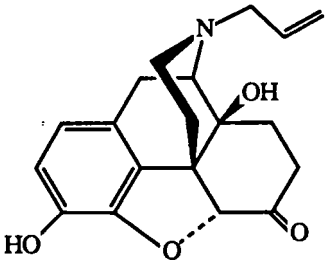
[a]slight ataxia

MONKEY DATA
(SDS)

At the highest dose (8.0 mg/kg), severe ataxia was noted in all monkeys receiving NIH 10556. In addition, one monkey vomited; developed jaw sag and appeared stuporous. However, as shown in the graph, this compound did not substitute completely for morphine. Most of the suppression of withdrawal signs at 8.0 mg/kg may be attributed to dimunition of response following abdominal palpation and to a decrease in restlessness.



NIH 10562, NIH 7890 Naloxone hydrochloride



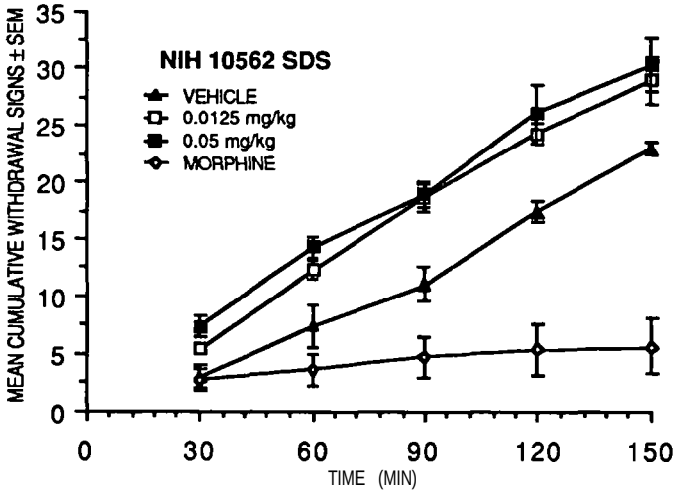
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - 0.03 (0.01 - 0.1)
3) PPQ - 1.3 (0.2 - 6.8)

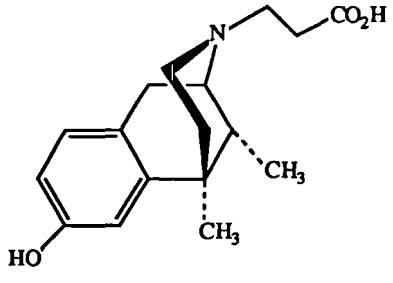
MONKEY DATA
(SDS)

As shown in the fig., NIH 10562, at 0.05 and 0.0125 mg/kg, exacerbated withdrawal. Onset of action was prompt and offset was greater than 150 min. It

should be noted that antagonists are much more potent and have a longer duration of action in withdrawn, morphine-dependent monkeys.



NH 10564 (±)-2-(2-Carboxyethyl)-5,9 α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

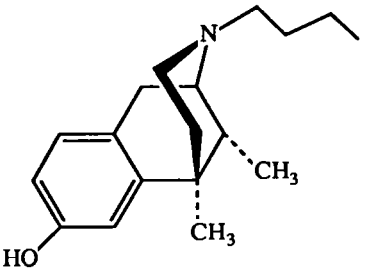


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - Inactive at 0.1, 1.0, 3.0, 10.0 and 30.0
3) PPQ - 1.4 (0.3 - 6.4)[a]

[a]Repeated: 16% at 0.3, 34% at 1.0, 61% at 5.0, 50% at 10.0 and 47% at 30.0

NIH 10565 (+)-2-Butyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride

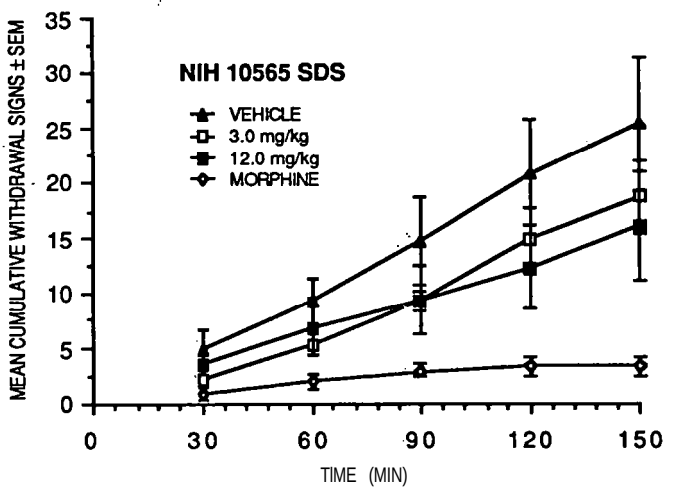


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

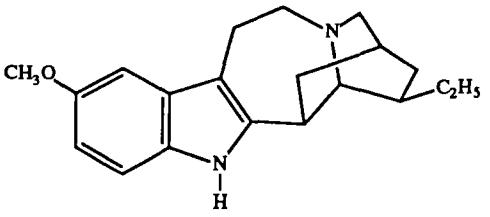
1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. Inactive at 1.0 and 30.0
3) PPQ - 23% at 0.1, 29% at 0.3, 43% at 1.0, 14% at 3.0, 29% at 10.0 and 43% at 30.0

MONKEY DATA
(SDS)

At the highest dose, two monkeys were ataxic. One of. the animals also appeared disoriented and had head tremor. In addition; some-of the monkeys at the high and low dose did not vocalize and had relaxed muscles when their abdomens were palpated. As indicated in the illustration, the drug did not substitute completely for morphine.



NIH 10567 Ibogaine hydrochloride



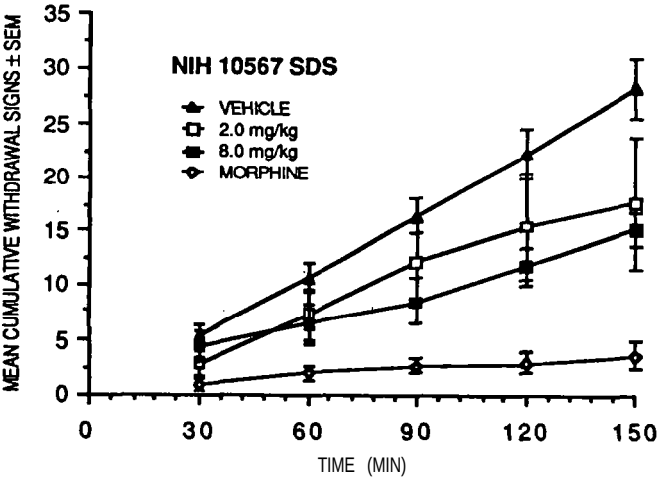
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF- Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9.7 (2.8 - 34.0)
4) HP - Inactive to 20.0

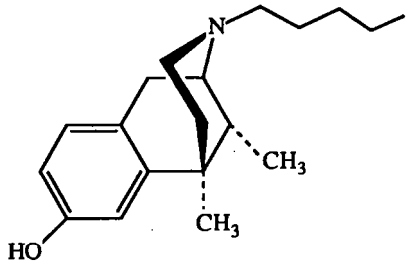
Vehicle - 2% Tween 80 in H_20

MONKEY DATA
(SDS)

As shown in the fig, NIH 10567 reduced the total number of withdrawal signs but did not substitute completely for morphine. Some of the monkeys, especially at the highest dose had relaxed abdominal muscles and did not vocalize when palpated. Partial substitution does not necessarily imply opioid activity. Severe tremors were noted at the highest dose.



NIH 10569 (-)-5,9α-Dimethyl-2'-hydroxy-2-pentyl-6,7-benzomorphan hydrochloride

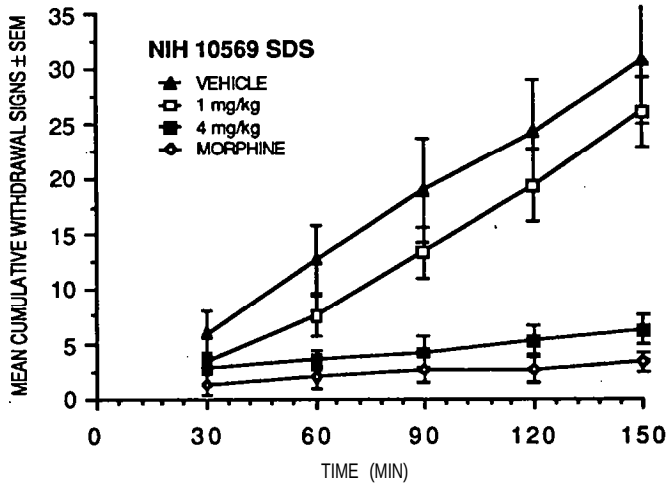


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

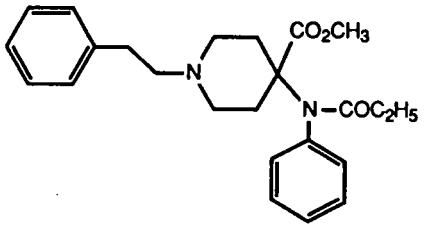
1) TF - 1.3 (0.7 - 2.4)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1) 0.2 (0.08 - 0.6)

MONKEY DATA
SDS

As shown in the fig., NIH 10569 substituted completely for morphine at the highest dose. The-drugs appears to be similar to morphine with respect to onset, duration of action and potency. However, one animal receiving the highest dose convulsed and another vomited 2 1/2 hr after drug.



NIH 10570 Carfentanil citrate



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg or % change)

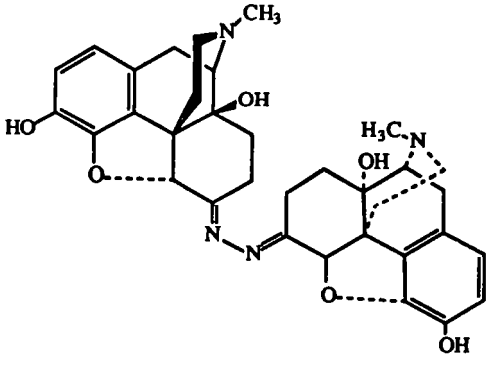
1) TF - 0.00021 (0.00008 - 0.005)[a]
2) TF vs. M. - Inactive at 0.0001 and 0.0003[a]
3) PPQ - 0.000058 (0.000029 - 0.00011)[a]
4) HP - <0.0004[a]

[a]Reported previously
See NIDA Monograph 90, 1988.

A. Special Study: Naloxone AD50 vs ED80 of morphine on Tail-Flick test of NIH 10570 when naloxone given after NIH 10570 was 0.02 (0.01 - 0.05)

B. Special Study: Naloxone AD_{50} vs ED80 of morphine on Tail-Flick when naloxone given before NIH 10570 was 0.035 (0.017 - 0.074).

NIH 10571 Oxymorphazine dihydrochloride



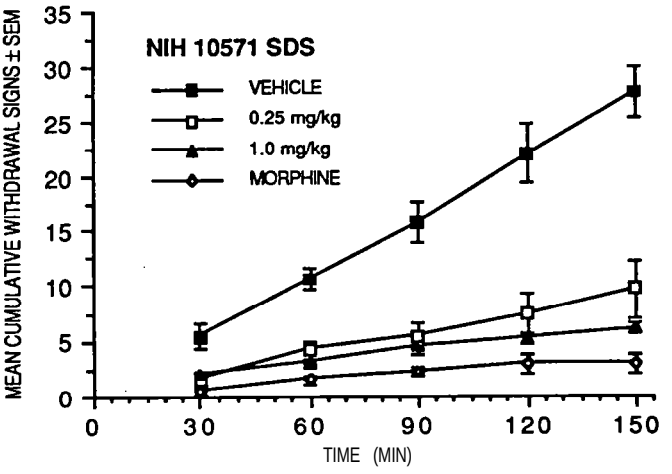
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg or % change)

1) TF - 0.09 (0.03 - 0.30)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.
3) PPQ - 0.03 (0.01 - 0.08)
4) HP - 3.1 (2.2 - 4.3)

Vehicle - Tween80 in H_2O

MONKEY DATA
(SDS)

Except for wet-dog shakes, NIH 10571 suppressed all other withdrawal signs in withdrawn, morphine-dependent monkeys. The drug is considered to be 5-10 x more potent than morphine and has a similar onset and offset of action (see fig.)



HIH 10573, NIH 7785 (±)-5.9α-Dimethyl-2'-hydroxy-2-pentyl-6,7-benzomorphan hydrochloride

See NIH 10569

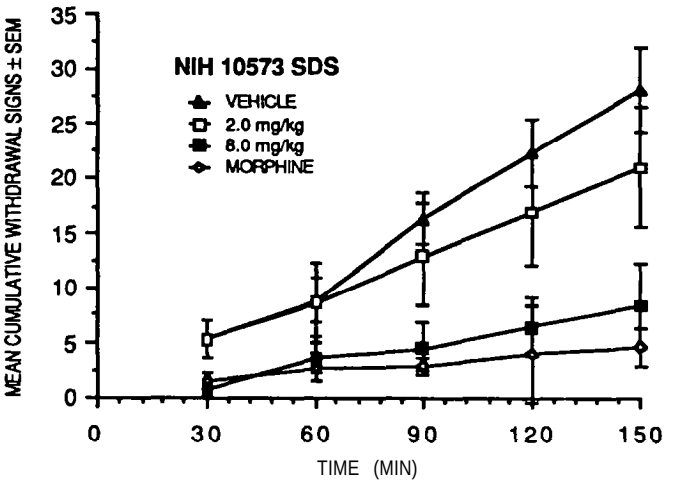
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.8 (0.3 - 2.0)
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.1 - 0.7)

MONKEY DATA

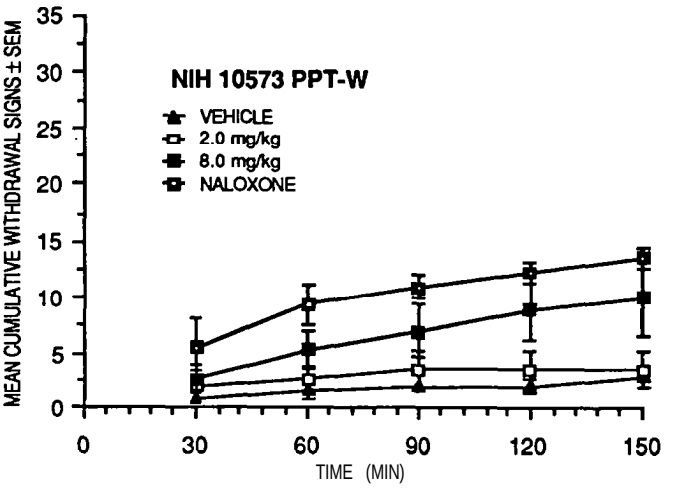
A. (SDS)

This drug produced some dose-related suppression of withdrawal signs in withdrawn, morphine-dependent monkeys. It appeared to have some agonist and antagonist effects depending upon whether the dependent monkeys were in withdrawal or not. It may be a partial mu agonist.

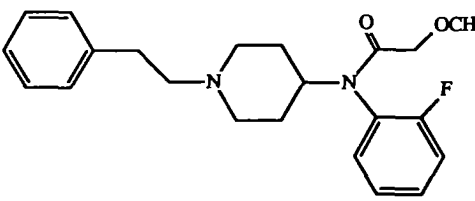


B. (PPt-W)

NIH 10573 produced a dose-related but low intensity withdrawal syndrome in non-withdrawn, morphine-dependent monkeys. At the highest dose, one monkey convulsed. Pentobarbital quickly suppressed these convulsions. The drug acted quickly and its duration of action was longer than that of naloxone. the positive control.



NIH 10579 1-(2-Phenylethyl)-4-[N-(2-fluorophenyl)methoxyacetamido]-piperidine hydrochloride

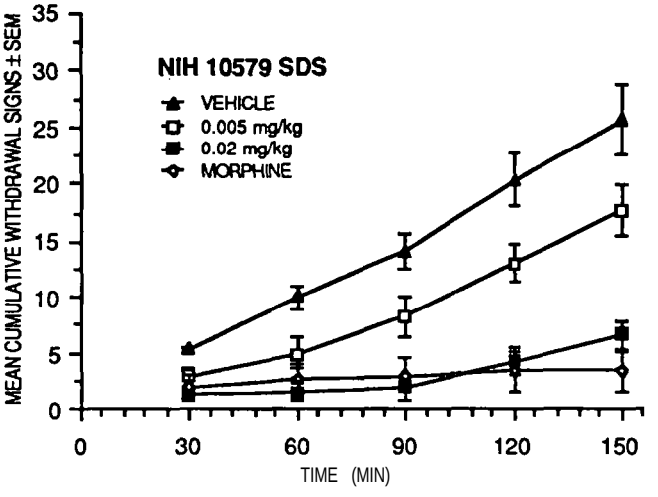


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

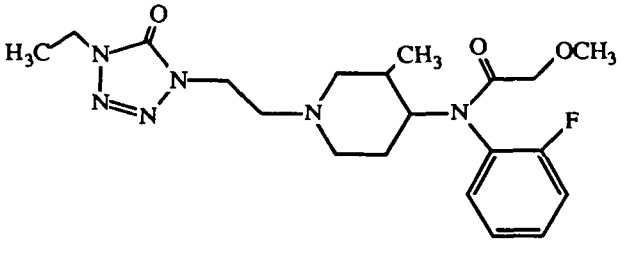
1) TF - 0.002 (0.0004 - 0.01)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.000075 (0.000045 - 0.00013)

MONKEY DATA
(SDS)

NIH 10579 substituted completely for morphine at both doses. Its onset of action is rapid and duration is about 90 min. The drug is approximately 1000 x more active than morphine (see accompanying fig.)



NIH 10580 1-[2-(4-Ethyl-4,5-dihydro-1H-tetrazolin-5-one-1-yl)ethyl]-3-methyl-4-[N-(2-fluorophenyl)methoxyacetamido]piperidine hydrochloride

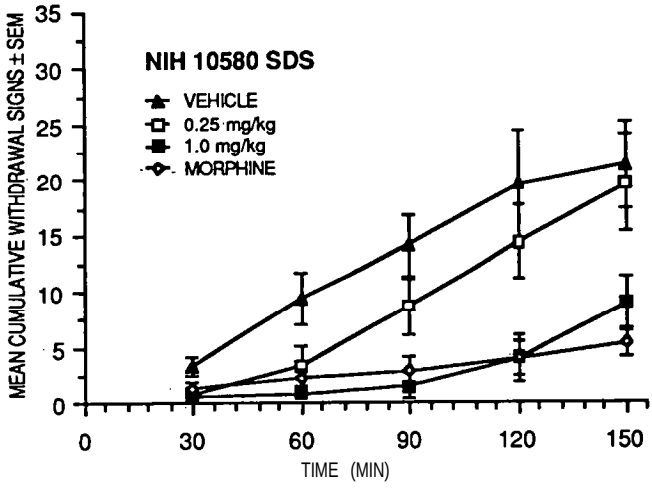


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

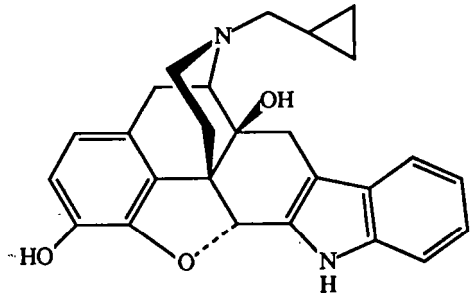
1) TF - 0.5 (0.2 - 0.9)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.04 (0.02 - 0.09)

MONKEY DATA
(SDS)

At the highest dose, NIH 10580 substituted completely for morphine. Onset and offset of action appear to be similar to morphine (see fig.). The drug is considered to be 1 or 2 times mote potent than morphine.



NIH 10589 Naltrindole hydrochloride

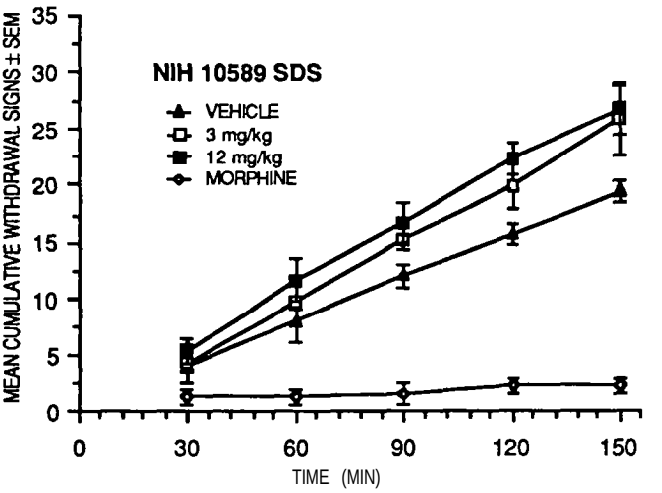


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

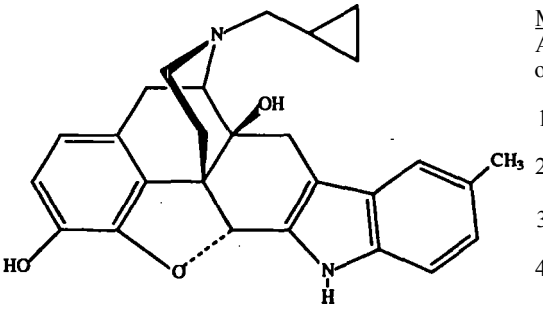
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs.M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0% at 1.0 and 10.0 and 28% at 30.0

MONKEY DATA
(SDS)

This compound did not substitute for morphine. It exacerbated withdrawal at both doses (see fig.). One animal became unusually aggressive towards the handler.



NIH 10590 Methyl naltrindole hydrochloride

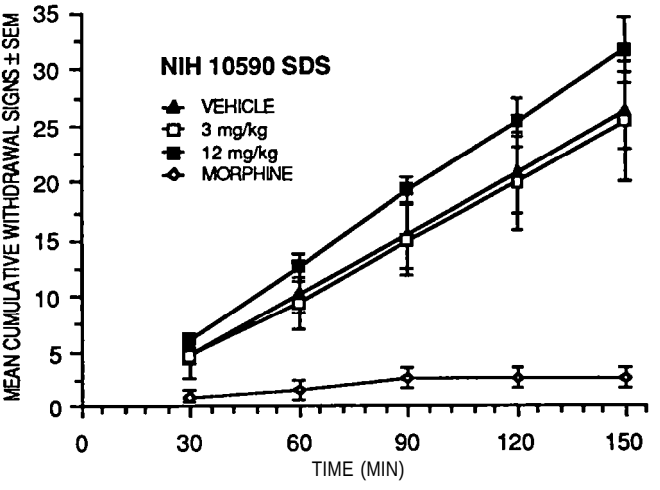


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

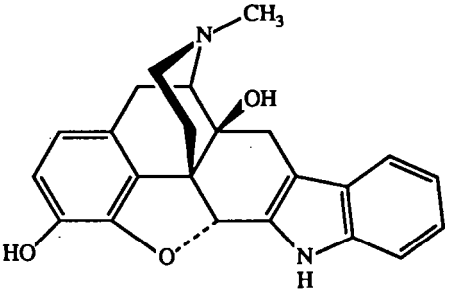
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 23% at 1.0, 11% at 10.0 and 20% at 30.0
4) H P -

MONKEY DATA
(SDS)

NIH 10590 did not substitute for morphine. Instead, it exacerbated withdrawal at the highest dose (see fig.). This compound is probably a very weak mu antagonist.



NIH 10591 N-Methyl-N-nomaltrindole hydrochloride

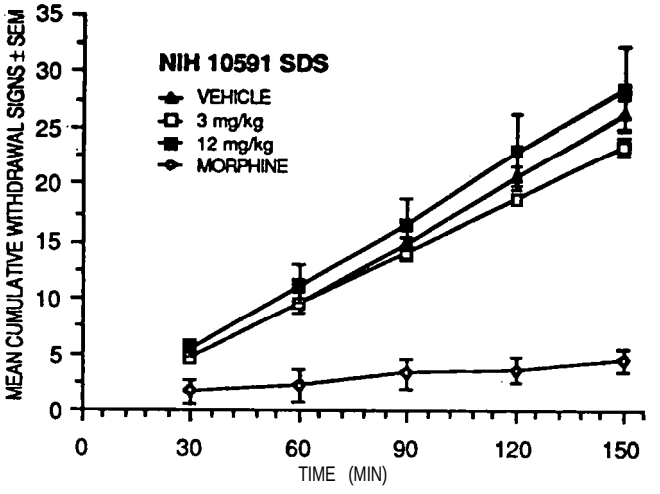


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

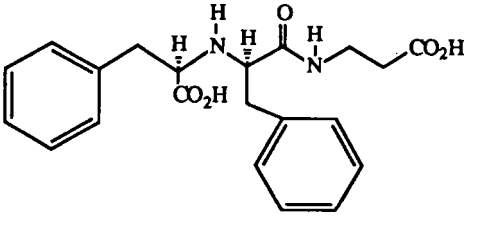
1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 46% at 1.0, 49% at 30.0 and 66% at 60.0

MONKEY-DATA
(SDS)

At the highest, dose, NIH 10591 exacerbated withdrawal in withdrawn, morphine-dependent monkeys. This compound appears to be a very weak mu antagonist.



NIH 10592 (S)-N-[(1-Carboxy-2-phenyl)ethyl]-(S)-phenylalanyl-ß-alanine



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg sc or % change)

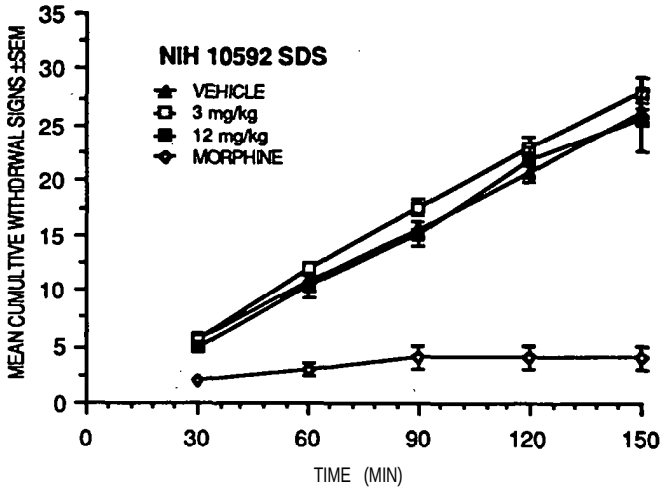
1) TF - 16% at 1.0, 8% at 10.0 and 3% at 30.0[a]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 27% at 0.5, 57% at 1.0, 59% at 5.0, 65% at 10.0 and 59% at 30.0[a]

[a]Vehicle - few drops of 2% $NaHC0_3$ solution, then H_20.

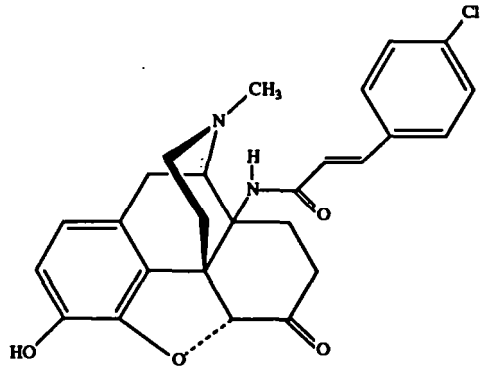
MONKEY DATA
(SDS)

At doses up to 12.0 mg/kg, NIH 10592 neither substituted for morphine nor exacerbated withdrawal. Drug supply exhausted. The drug was dissolved in 2% $NaHC0_3$ solution, and then, H_20 was added.

268-365 0 - 90 - 21 : QL 3



NIH 10594 mesylate — 14ß(*p*-Chlorocinnamoylamino)-7,8-dihydromorphinone



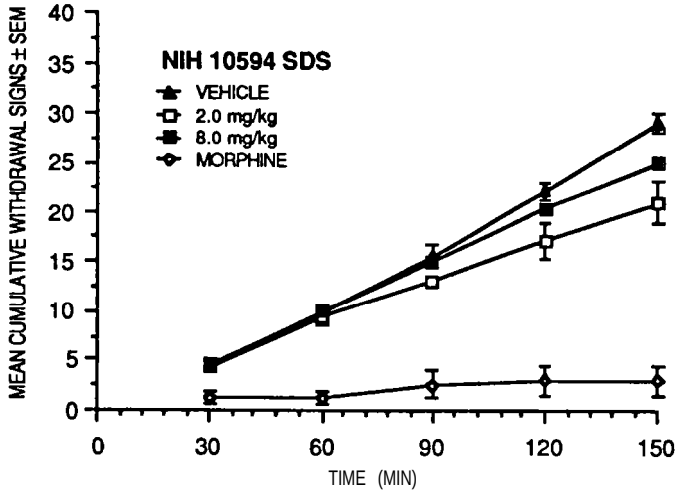
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 12% at 1.0, 9% at 10.0 and 22% at 30.0[a]
2) TF vs. M. - 3.8 (1.2 - 11.7)[a]
3) PPQ - 1.2 (0.3 - 4.8)[a]

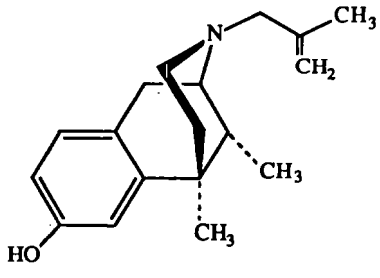
[a]Vehicle -Lactic acid and H_20

MONKEY DATA
(SDS)

NIH 10594 did not substitute for morphine. At the highest dose, one monkey had a seizure. Drug supply exhausted.



NIH 10595 (-)-5.9α-dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M. - 0.8 (0.7 - 1.0)
3) PPQ - 0.8 (0.2 - 3.1)[b]

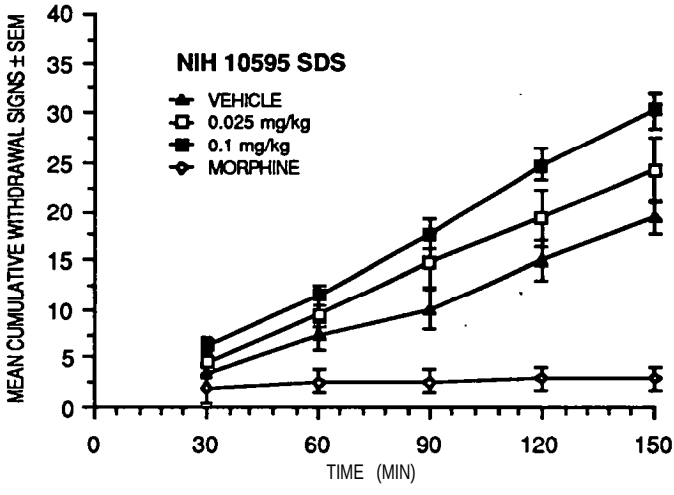
[a]Severe ataxia, shaking, and loss of righting reflexes at 30.0

[b]Straub tail, ataxia, hyperactivity at 10.0

MONKEY DATA

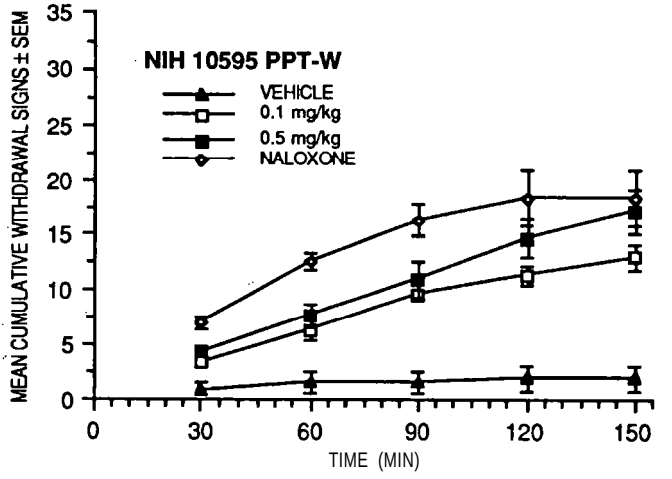
A. SDS

The drug did not substitute for morphine. Instead, it promptly exacerbated withdrawal in a dose-wise fashion (see fig.).

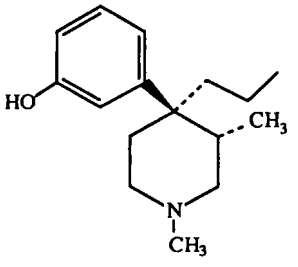


B. PPt-W

NIH 10595 precipitated withdrawal. The drug acted promptly and the duration of action was at least 2 1/2 hrs much longer than that of naloxone (60-90 min). Potency is estimated as 1/100 that of naloxone at peak effect. In addition, at the highest dose. jaw and body sag, ataxia and eyelid ptosis were noted. The monkeys also seemed to be disoriented at this dose.



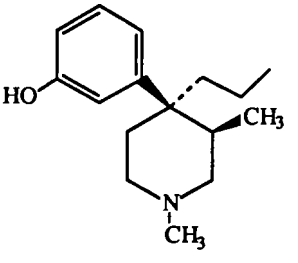
NIH 10596 4-(3-Hydroxyphenyl)-1,3--dimethyl-4- *n*-propylpiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 1% at 1.0, 30% at 3.0, 59% at 10.0 and 62% at 30.0
2) TF vs. Morphine - 0% at 1.0 and 3.0, 32% at 10.0 and 13% at 30.0
3) PPQ - 1.4 (0.6 - 3.0)

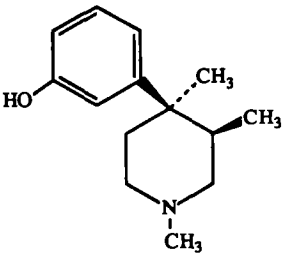
NIH 10597 4-(3-Hydroxyphenyl)-1,3-dimethyl-4- *n*-propylpiperidine hydroxhloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 17% at 1.0, 42% at 3.0, 53% at 10.0 and 36% at 30.0
2) TF vs. M. - 6.1 (2.6 - 14.2)
3) PPQ - 2.9 (1.1 - 7.7)

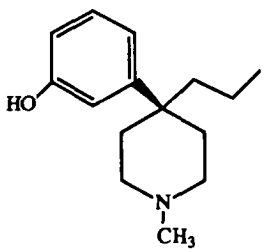
NIH 10596 4-(3-Hydroxyphenyl)-1,3,4-trimethylpiperidine (*cis* aryl-methyl) hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. Morphine - 1.2 (0.4 - 3.5)
3) PPQ - 11% at 1.0, 22% at 10.0 and 22% at 30.0

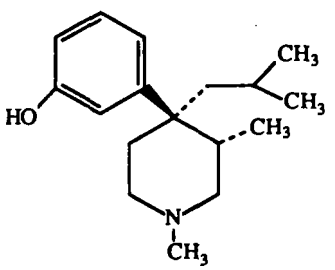
NIH 10599 4-(3-Hydroxyphenyl)-4-*n*-propyl-l-methylpiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.5 (0.1 - 1.9)
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.2 - 0.9)

NIH 10600 4-(3-Hydroxyphenyl)-1-3dimethyl-4-(2-methyl-prop-1-yl)piperidine (*trans* aryl-methyl) hydrochloride

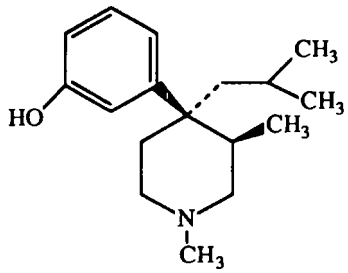


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 2.3 (1.2 - 4.5)[a]
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.1 - 0.6)

[a]Straub tails

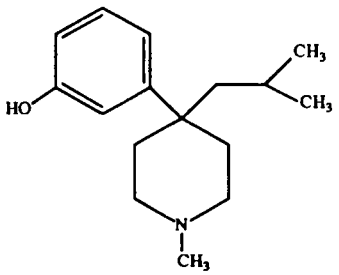
NIH 10601 4-(3-Hydroxyphenyl)-1-3-dimethyl-4-(2-methyl-prop-1-yl)piperidine (*cis* aryl-methyl) hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 17.6 (12.0 - 26.2)
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.7 (0.5 - 5.8)

HIV 10602 4-(3-Hydmxyphenyl)-4-isobutyl-1-methylpiperidine hydrochloride

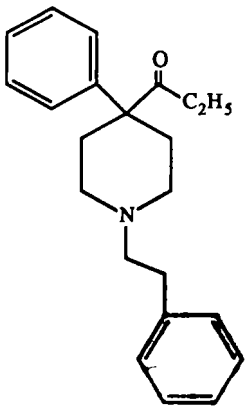


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 0.8 (0.3 - 2.1)
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ- 0.3 (0.1 -0.7)

[a]Straub tails at 30.0 - 1 of 6 died shortly after experiment.

NIH 10603 1-Phenethyl-4-phenyl-4-propionylpiperidine hydrochloride

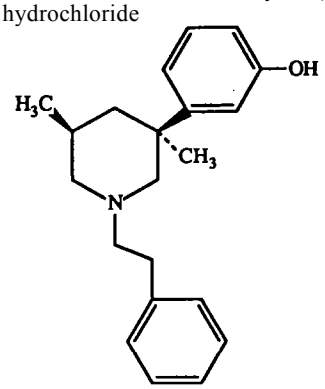


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 6.4 (2.3 - 18.0)[a]
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 6.3 (3.4 - 11.4)
4) H P -

[a]Straub tail

NIH 10604 3,5-Dimethyl-3-(3-hydroxyphenyl-1-phenethylpiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 4.7 (1.5 - 15.0)

[a]Decreased reaction time and tremor and convulsions in 3 animals.

NIH 10608 (+)-5,9α-Dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride

MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

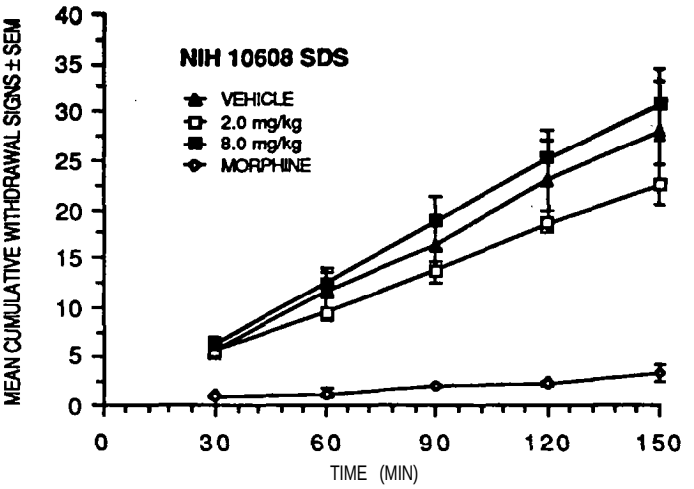
See NIH 10595

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. Morphine - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 18.6 (12.0 - 29.0)

[a]2 hr pretreatment - 14% at 30.0

MONKEY DATA
(SDS)

NIH 10608 neither substituted for morphine nor exacerbated withdrawal (see fig.). The apparent attenuation of withdrawal at the low dose (2.0 mg/kg) was due to a reduced incidence of the signs designated as lying on side, restlessness and vocalization. Also, no vomiting was noted at the low dose.



NIH 10612 (±)-5,9α-Dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride

See NIH 10595

MOUSE DATA-ED OR AD50 95% C.L.) (mg/kg or % change)

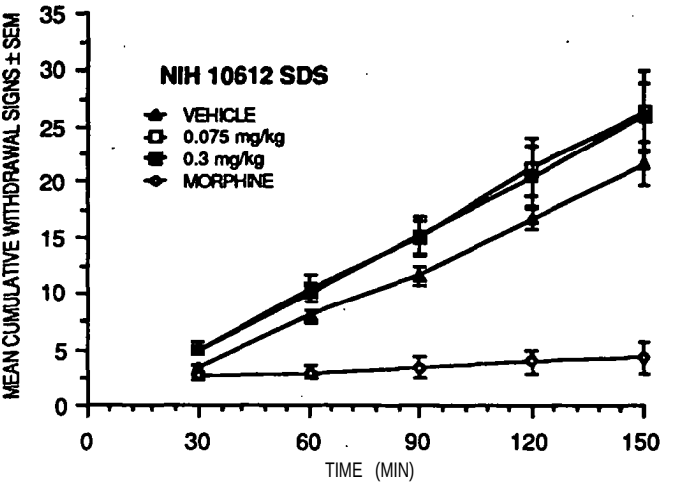
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. Morphine - 0.9 (0.3 - 2.6)[b]
3) PPQ - 1.8 (0.5 - 6.1)

[a]Severe ataxia and vocalization, decrcase tail-flick latencies at 10.0 and 30.0.
[b]ataxia

MONKEY DATA

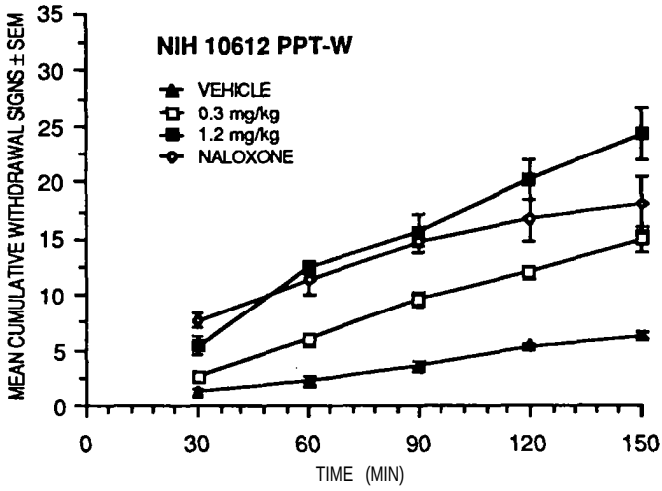
A. (SDS)

As shown in the illustration, NIH 10612 did not substitute for morphine and may have exacerbated withdrawal at doses of 0.3 and 0.75 mg/kg. At the highest dose, the signs ataxia, slowing and jaw sag also were noted.

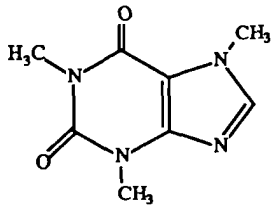


B. (PPt-W)

NIH 10612 precipitated withdrawal in a dose-related manner. Rapid onset and long duration (at least 2 1/2 hr) characterized the drug. The duration of action of naloxone was approximately 90 min. Because of the dissimilar offset of action times for NIH 10612 and naloxone. the reference standard, the potency estimate cannot be reliably estimated.



NIH 10613 Caffeine and Sodium Benzoate (U.S.P. XIV)

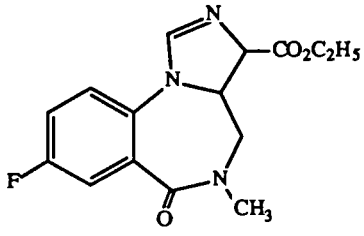


MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

1) TF - 106.9 (77.00 - 148.37)
2) TF vs. M. - Inactive at 1.0, 10.0, 30.0 and 60.0
3) PPQ - Inactive at 1.0. 10.0, 30.0 and 60.0[a]

[a]Dose expressed as base

NIH 10616 8-Fluoro-5,6-dihydro-5-methyl-6-oxo-4 *H*-imidazo[1,5a][1,4]-benzodiazepine-3carboxylate ethyl ester (Ro 15-1788). (Flumazenil)



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

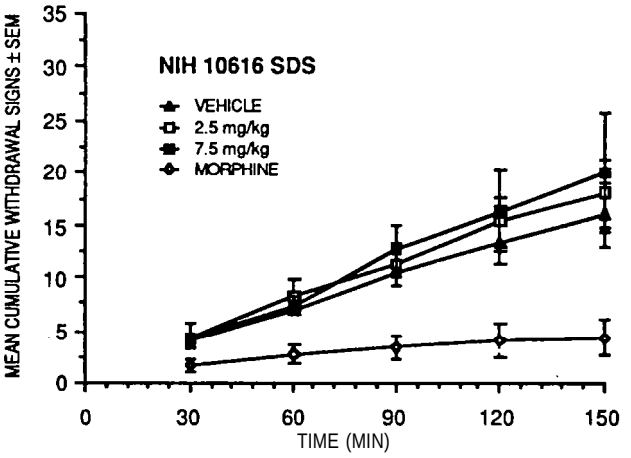
1) TF - Inactive at 0.01, 0.05, 0.1, 1.0, 10.0 and 30.0[a,b]
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0[b]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[b]
4) HP -

[a]Vocalization, decreased latencies in TF test in 4 of 6 mice at 1.0, VEHICLE: EtoH, propylene glycol and H_20 (1: 4: 5)

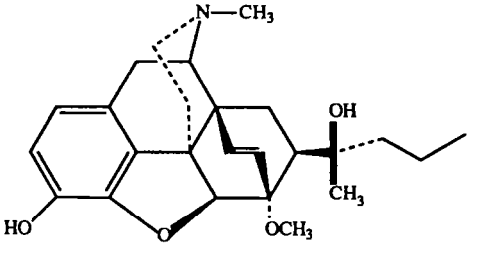
between 80 and H_20

MONKEY DATA
(SDS)

NIH 10616, a benxodiazepine antagonist and inverse agonist-antagonist, neither substituted for morphine nor exacerbated withdrawal at 2.5 and 7.5 mg/kg. Vehicle consisted of ethanol, propylene glycol and water (1: 4: 5).



NIH 10617 (+)-Etorphine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg or % change)

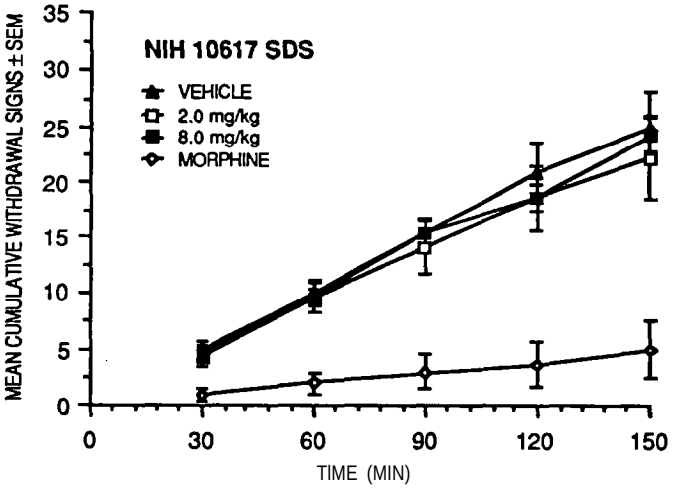
1) TF - Inactive at 0.01, 0.05, 1.0, 10.0 and 30.0[a]
2) TF vs. M. - 1% at 0.1, 19% at 0.3, 36% at 1.0, 0% at 3.0, 9% at 10.0 and 5% at 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP -

[a]Decreased test latencies in tail-flick test

MONKEY DATA

(SDS)

As shown in the accompanying fig., NIH 10617 neither substituted for morphine nor exacerbated withdrawal at the doses tested. At the highest dose, frequent vomiting was noted in 2 of 3 monkeys.



ACKNOWLEDGEMENTS

This study was supported by a contract (#271-87-8116) from the National Institute on Drug Abuse, Dr. Geralin Lin, Contract Officer. We also acknowledge the expert assistance of Susan M. Tucker and Christopher C. Cull. Special thanks to Dr. Billy R. Martin, Ramona Winckler and Laura Johnson for their help in the preparation of this manuscript using the Macintosh Plus computer.

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1989 Annual Report, Evaluation of New Compounds for Opioid Activity

James H. Woods, Fedor Medzihradsky, Charles B. Smith Gail D. Winger and Charles P. France

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia is coordinated by Dr. Arthur E. Jacobson, Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universites, government laboratories, and international organizations are submitted to Dr. Jacobson, who performs the MOUSE ANALGESIA tests. Values obtained in these tests for some representative opioid drugs are given in Table I.

At the UM and MCV laboratories, drug samples arrive from Dr. Jacobson with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information and (4) a recommended starting dose. After the evaluation is complete and the report submitted to Dr. Jacobson, the submitter is requested to release the chemical structure to include with the evaluation data in the ANNUAL REPORT. The submitter has up to three years before release of the structure is required. When the structure is released all of the data on the compound are reported to the Committee.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use two groups of monkeys to test the discriminative effects of submitted drugs. One of these groups is trained to discriminate the administration of the kappa agonist ethylketazocine (EKC). The other group is trained to discriminate the mu agonist, codeine.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al. (1982). The monkeys are 'removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in isolation chambers equipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the trial.

The left lever is designated correct if they were given a sham injection before the start of the trial. Each trial lasts 15-min and consists of an initial 10-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are earned before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min trials. During a training session, if EKC is given, it is given on the penultimate trial of that session. Responding on the drug-appropriate lever is reinforced during that trial and on the subsequent, final trial of the day. These last two trials may be preceded by from zero to four sham trials on a training day. A training session of six sham trials is also scheduled from time to time.

With this type of multiple, discrete-trial training, the animals can be tested with a cumulative dosing procedure. On a test session, the first trial is preceded by an injection of saline, and prior to subsequent trials, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six trials are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each trial of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the codeine-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-trial paradigm. The main difference between the codeine procedure and the EKC procedure is that the codeine monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can earn as many as 10 pellets during the five minute, food-availability period of each trial, but each pellet is earned by making 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 40 total responses prior to earning the first food pellet of each trial. Tests of the discriminative effects of submitted drugs in the codeine-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

The single-dose suppression (SDS) test determines the ability of a drug to suppress the signs of withdrawal in monkeys which have been made dependent by the chronic administration of morphine (3 mg/kg every six hours). Compounds suspected of having morphine-antagonist properties are tested for their ability to precipitate the withdrawal syndrome in nonwithdrawn (NW) morphine-dependent monkeys. Nondependent monkeys (Normals) are used to determine whether the acute effects of the test drug are reversible by nalorphine or naloxone. In a primary dependence (PDS) study, non-dependent monkeys receive the test drug every six hours for 30 days to determine whether withdrawal signs will appear when the animals are challenged with an antagonist or when drug administration is discontinued.

Details of these techniques have been presented in the ANNUAL REPORT to the Committee in 1963 (Minutes of the 25th Meeting) by Deneau and Seevers (1963) and by Villarreal (1973).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce a five-sec intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition is in effect, during which responses have no scheduled consequence and neither light is illuminated. Each of the two daily sessions consist of 13 injections or 130 min, whichever occurs first. Other details of the procedure and initial findings with a variety of narcotics are given in previous reports (e.g., Woods, 1977; 1980).

Doses of the drugs are typically described in terms of mg/kg/injection (inj). Duplicate observations of codeine (0.32 mg/kg/inj) and of saline are obtained for each monkey. A saline substitution is conducted before and after the series of observations on a test drug; the control rates of codeine-reinforced responding are obtained by a random sampling of two sessions interpolated between the drug-substitution sessions. These data are represented in the following graphs with individual symbols for each of the monkeys; each symbol is the mean of duplicate observations for a given dose in each monkey. The closed circles indicate the averaged data for observations on the subset of monkeys used to study each drug under each of the experimental conditions. In all cases, the rates of responding given are those calculated during only the fixed-ratio portion of each session.

DISPLACEMENT OF RADIOLABELED LIGAND BINDING

Details of the binding assay, based on the displacement of ^{3}H-etorphine in rat brain membranes have been described previously (Medzihradsky et al., 1984). Briefly, aliquots of a membrane preparation from rat cerebrum are incubated with ^{3}H-etorphine in the presence of 150 mM NaCl, and in the presence of different concentrations of the drug under investigation. Specific, i.e., opioid-receptor-related interaction of ^{3}H-etorphine is determined as the difference in binding obtained in the absence and presence of an appropriate excess of unlabeled etorphine. The potency of the drugs in displacing the specific binding of ^{3}H-etorphine is determined from log-probit plots of the data. It should be noted that since April 1982 the concentration of ^{3}H-etorphine in the binding assay was reduced from 3.0 nM to 0.5 nM, a concentration approaching the KD of the radiolabeled opioid. This change was implemented in order to let the determined EC50 approximate the true Ki of a given drug. As a consequence of the different concentration of the radiolabeled ligand, the EC50 determined since April, 1982 are lower than those obtained previously. For the purpose of reference, Table II contains EC50 values of representative opiates determined in binding assays using 0.5 nM and 3.0 nM ^{3}H-etorphine.

To enhance the characterization of newly synthesized opiates, we are now investigating their selectivity in binding to mu-, delta-, and kappa-opioid receptors in membranes from monkey brain cortex. Thus, we are now providing EC50 values of the tested compounds in displacing the following radiolabeled opioid ligands:

etorphine (nonselective, reflects opioid character),

sufentanil or Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (mu selective),

[D-Pen^{2}-D-Pen^{5}]enkephalin (delta selective),

U-69,593 (kappa selective).

Using the receptor-specific assays, we have described the selectivity of various established opiates in brain membranes of different species (Clark et al., 1988). The selection of monkey brain as the tissue for the selective binding assays strengthens the correlation between this in vitro assessment and the behavioral evaluation of the tested compounds. In the ANNUAL REPORT, the results of the selective binding assays are listed under "Binding in monkey brain cortex".

Within our goal to enhance the molecular characterization of novel opiates (Medzihradsky, 1987) we have established a functional assay for receptor-effector interaction, reflecting receptor coupling to regulatory G protein. The method is based on the stimulation of brain GTPase by opioid agonists, a process blocked by antagonists (Clark and Medzihradsky, 1987). We are

presently evaluating the quantitative responses of partial agonist-antagonists in this assay. Considering the variable efficacy of opioid receptor occupancy (Clark et al., 1989) the new assay provides a functional parameter in the characterization of novel opiates, distinguishing thereby between agonists and antagonists.

INHIBITION OF TWITCH IN ELECTRICALLY-STIMULATED MOUSE VAS DEFERENS PREPARATIONS.

The development of new, highly selective antagonists such as the irreversible mu receptor antagonist beta-funaltrexamine (beta-FNA) and the reversible delta receptor antagonist ICI-174864 have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse vas deferens preparation. Male, albino ICR mice, weighing between 25 and 30 g, are used. The mice are decapitated, the vasa deferentia removed, and 1.5 cm segments are suspended in organ baths which contain 30 ml of a modified Kreb's physiological buffer. The buffer contains the following (mM): NaCl, 118; KCl, 4.75; $CaC1_2$. 2.54; $MgS0_4$, 1.19; KH_2P0_4, 1.19; glucose, 11; $NaHC0_3$, 25; pargyline HCl, 0.3, tyrosine, 0.2; ascorbic acid, 0.1; and disodium edetate. 0.03. The buffer is saturated with 95% 02 - 5% CO_2 and kept at 37° C. The segments are attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments are stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage.

The following antagonists are studied: naltrexone HCl, ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and beta-FNA. Naltrexone and ICI-174864 are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. Beta-FNA is added to the organ baths after the initial equilibration period. Thirty min later, the beta-FNA is removed from the organ baths by repeated washings with fresh buffer. The tissues are washed three times every 5 min for 30 min. Cumulative concentration-effect relationships for the various agonists are then determined 10 min after the last wash (i.e., 30 min after the beta-FNA was removed from the organ baths). EC50's are calculated by probit analysis, and pA_2 values are determined to assess relative potencies of antagonists. All drugs which are submitted for evaluation are studied in the following manner: 1) the submitted drug is tested on the vas deferens preparation in the absence and in the presence of naltrexone. The concentration of the unknown drug is varied from the lowest with activity to that which is maximally effective. 2) If the submitted drug inhibits the twitch, the ability of naltrexone to reverse the inhibition is determined. 3) The submitted drug is assessed for its ability to antagonize the actions of morphine on the vas deferens. 4) The drug is assessed for its ability to reverse the inhibition produced by a maximally effective concentration of morphine. 5) Finally, if the drug has opioid agonistic activity, studies-are conducted to determine the receptor type upon which it acts. If it has antagonistic activity upon the vas deferens or upon any of the

other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive) and the receptor selectivity is determined, For further details of the procedure see Smith (1986). Drugs studied in the preparation prior to 1987 were evaluated with the protocol reported in the 1985 Annual Report.

TABLE I

MOUSE ANALGESIA. Before submission to The University of Michigan, all compounds are evaluated for analgesic activity by Dr. Arthur E. Jacobson. Shown below are comparative data (ED 50mg/kg) (95% Confidence Interval) from Hot Plate[a-c] and Nilsen[d] assays. umol/kg

Compound	HOT PLATE (sc/mg/kg)	HOT PLATE (oral,mg/kg)	NILSEN (sc, mg/kg)	NILSEN (oral, mg/kg)
NIH #	(sc, umol/kg)	(oral, umol/kg)	(sc. umol/kg),,	(oral, umol/kg)
Morphine sulfate	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-1.7)	8.3 (6.0-11.4)
NIH 0001, 9929	2.9 (2.5-3.3)	18.9 (14.1-24.9)	3.9 (3.0-5.1)	24.9 (18.0-34.1)
Codeine phosphate	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
NIH 0002	17.1 (11.3-25.7)	34.0 (24.4-47.1)	18.6 (12.3-27.7)	37.0 (23.2-58.7)
Levorphanol tartrate	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
NIH 4590	0.5 (0.2-0.7)	-	0.5 (0.4-0.7)	6.2 (4.2-9.1)
Meperidine.HCl	5.3 (4.0-7.1)	-	-	-
NIH 5221	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
NIH 7569	1.9 (1.4-2.8)	34.1 (25.7-45.3)	1.6 (1.0-2.3)	83.6 (67.5-106.1)

TABLE I Continued

Dihydromorphinone.HCl NIH 0123	0.19 (0.15-0.25)	0.9 (0.7-1.2)	0.2 (0.15-0.3)	1.8 (1.5-2.1)
	0.6 (0.5-0.8)	2.8 (2.2-3.7)	0.6 (0.5-0.9)	5.6 (4.7-6.5)
Nalorphine.HCl NIH 2105	9.9 (5.7-17.1)	-	23.0 (16.2-32.7)	-
	28.4 (16.4-49.1)	-	66.1 (46.6-94.0)	-
Cyclazocine NIH 7981	1.5 (1.1-2.1)	-	0.1 (0.07-0.16)	-
	5.5 (4.1-7.7)	-	0.4 (0.3-0.6)	-
Pentazocine NIH 7958	9.3 (6.7-12.8)	-	6.5 (4.4-8.8)	-
	32.6 (23.5-44.9)	-	22.8 (15.4-30.9)	-
Naltrexone.HCl NIH 8503			No dose response	
Naloxone.HCl NIH 7890			No dose response	
No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.				
Chloropromazine.HCl	1.1 (0.9-1.5)			
	3.2 (2.4-4.2)			

a) Eddy and Leimbach (1953); b) Jacobson and May (1965); c) Atwell and Jacobson (1978); d) Perrine, Atwell, Tice, Jacobson and May (1972).

TABLE II

EC50's of representative opioids for displacement of ^{3}H-etorphine from rat brain membrane, and inhibition of the twitch of the mouse vas deferens preparation.

	BINDING (+ 150 mM NaCl)		MVD
	EC50		
Compound	3H-etorphine (0.5 nM)	^{3}H-etorphine (3.0 nM)	EC50 (nM)
DPDPE	---	---	5.52
U50,488	---	---	6.29
Fentanyl	---	---	37.1
DAGO	---	---	81.3
Etorphine	0.37	4.2	0.0068
(-)Cyclazocine	0.53	3.40	11.9
Naltrexone	0.63	2.34	---
Bremazocine	1.42	---	---
-UM 1071R*	1.55	4.71	---
Sufentanil	1.60	---	4.43
(-)SKF 10047	3.93	20.5	---
Ethylketazocine	6.60	19.3	11.6
Ketazocine	14.1	63.1	1.18
Morphine	23.6	140	395
DSLET	43.0	---	1.71
Dextrorphan	9820	18000	1010

* lR-5R-9R-2"R-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan hydrochloride

SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year, and the individual tests which were performed are shown in Table III. Also shown are dates of Annual Reports in which results are reported of earlier tests on those compounds conducted at Michigan.

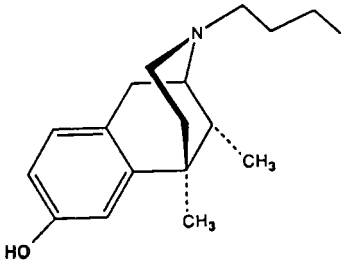
TABLE III

SUMMARY OF TESTS PERFORMED

NIH	CHEMICAL CLASS AND/OR GENERIC NAME	SA	MVD	BIND	DD	REPORT*
7589A	Benzomorphan	-	+	+	-	03/30/89
9994	Morphine	-	+	+	-	10/17/88
9995	Morphine	-	+	+	-	01/12/89
10168	Benzomorphan	-	+	+	-	10/17/88
10447	Piperidine-thiopyridine	-	+	+	-	04/03/86
10448	Piperidine-thiopyridine	-	+	+	-	04/03/86
10496	Phenylpiperazinylpyradazine	+	-	-	+	04/04/89
10497	Morphine	+	+	+	+	12/08/88
10501	Ethenoisomorphinan	-	+	+	-	10/17/88
10526	Phenylpiperidine	-	+	+	-	10/17/88
10544	Morphinone	-	+	+	-	10/30/87
10545	Morphinone	-	+	+	-	10/30/87
10567	Ibogaine	-	+	+	-	12/23/87
10571	Oxymorphazine	-	+	+	-	01/13/88
10572	Naltrexone, esterone	-	+	+	-	10/17/88
10573	Benzomorphan	-	+	+	-	10/17/88
10576	Phenylvaleramide	-	+	+	-	10/17/88
10579	Phenylvaleramide	-	+	+	-	10/17/88
10580	Tetrazolyl-piperidine	-	+	+	-	10/17/88
10589	Naltrindole	-	+	+	-	01/12/89
10590	Methylnaltrindole	-	+	+	-	01/12/89
10591	Oxymorphindole	-	+	+	-	01/12/89
10592	Phenylalanyl-alanine	-	+	+	-	03/30/89
10594	Morphinone	-	+	+	-	10/17/88
10595	Benzomorphan	-	+	+	-	10/17/88
10597	Phenylpiperidine	-	+	+	-	10/17/88
10598	Phenylpiperidine	-	+	+	-	10/17/88
10599	Phenylpiperidine	-	+	+	-	10/17/88
10600	Phenylpiperidine	-	+	+	-	10/17/88
10601	Phenylpiperidine	-	+	+	-	03/30/89
10602	Phenylpiperidine	-	+	+	-	03/30/89
10603	Phenylpiperidine	-	+	+	-	11/23/88
10605	Naloxone	-	+	+	-	01/12/89
10606	Naloxone	-	+	+	-	01/12/89
10607	Naloxone	-	+	+	-	01/12/89
10608	Benzomorphan	-	+	+	-	01/12/89
10612	Benzomorphan	-	+	+	-	01/12/89
10617	Etorphine	-	+	+	-	03/30/89
10620	Thevinone	-	+	+	-	03/30/89
10621	Thevinol	-	+	+	-	03/30/89

* Date report was submitted to CPDD Biological Coordinator.

NIH 7589A (±)-2-n-Butyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 101 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

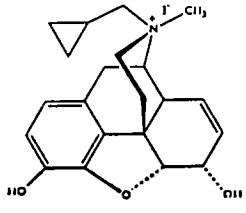
NIH 7589A studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.43 ± 0.23	1.5
DSLET	6.59 ± 0.63	1.7
U50,488	6.96 ± 0.65	1.8

SUMMARY

NIH 7589A had similar affinities in the two preparations; the significant deviations from unity for the Schild plots in the vas deferens suggests that it might not exert its actions as a simple, competitive antagonist.

NIH 9994 N-Methyl-N-cyclopropylmethylnormorphinium iodide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 30% at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 49.3 nM in the presence of 150 mM NaCl.

NIH 9994 N-Methyl-N-cyclopropylnormorphinium iodide

... (continued)

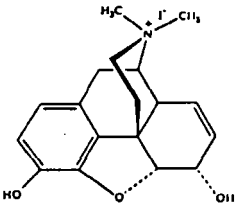
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.32×10^{-6}	35.1%
After naltrexone	4.84×10^{-6}	18.9%
With equimolar concentration of naltrexone	Complete reversal	

SUMMARY

NIH 9994 was more potent in the binding assay than in the vas deferens. It had opioid actions in both preparations. The compound was studied in 1982 in the smooth muscle preparation and only recently in the binding assay.

NIH 9995 N-Methylmorphinium iodide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive to 100

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2740 nM in the presence of 150 mH NaCl.

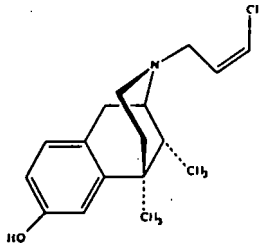
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.77×10^{-7}	55.1%
After naltrexone	6.08×10^{-8}	46.9%
With equimolar concentration of naltrexone	Slight reversal	
Equimolar concentration with morphine	No reversal	

SUMMARY

NIH 9995 had no significant opioid activity in either in vitro preparation.

NIH 10168 (-)-2-(cis-3-Chloroallyl)-5,9 α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 20% at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2.72 nM in the presence of 150 mM NaCl.

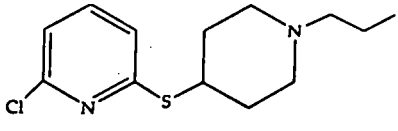
MOUSE VAS DEFERENS PREPARATION

	Inhibitory ECSO. (Ml	Maximum Response
Drug alone	2.60×10^{-8}	44.6%
After naltrexone	4.14×10^{-8}	60.3%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration. with morphine	Partial reversal	

SUMMARY

NIH 10168 had- opioid actions in both preparations; it would appear to be more potent in the binding assay, and it might be a partial agonist in the vas deferens.

NIH 10447 2-Chloro-6-(4-(N-n-propyl)piperidino)thiopyridine hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive to 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6000 nM (9.0% at 6000 nM) in presence of 150 mM NaCl.

NIH 10447 2-Chloro-6-(4-(N-n-propyl)piperidino)thiopyridine hydrochloride

...(continued)

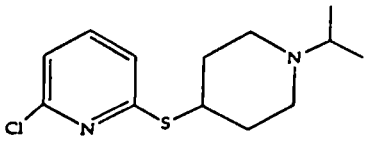
MOUSE VAS DEFERENS PREPARATION

With increasing concentrations, NIH 10447 caused a gradual inhibition of the twitch which was maximal at a concentration of 3×10^{-5} M. The maximum response was a 33.7% inhibition of the twitch. Responses to NIH 10447 were not altered in the presence of naltrexone (10^{-7} M). NIH 10447 was studied as an antagonist at a concentration of 10^{-7} M. It caused a parallel shift to the right (a 6.4-fold shift) in the sufentanil concentration-effect curve. In the absence of NIH 10447, the EC50 for sufentanil was 3.47 nM ± 1.42 and the maximum response was a 100% inhibition of the twitch. In the presence of NIH 10447, the EC50 for sufentanil was 22.3 nM ± 5.9 and the maximum response was a 100% inhibition of the twitch.

SUMMARY

NIH 10447 appears to be an opioid antagonist on the mouse vas deferens preparation, but it has no significant affinity for the etorphine binding site.

NIH 10448 2-(Chloro-6-(4-N-isopropylpiperidino)thiopyridine oxalate



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive to 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (18.6% at 6,000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

With increasing concentrations, NIH 10448 caused a gradual inhibition of the twitch which was maximal at a concentration of 3×10^{-5} M. The maximum response was a 42.9% inhibition of the twitch. Responses to NIH 10448 were not altered in the presence of naltrexone (10^{-7} M). NIH 10448 was studied as an antagonist

NIH 10448 2-(Chloro-6-(4-N-isopropylpiperidino)thiopyridine oxalate

... (continued)

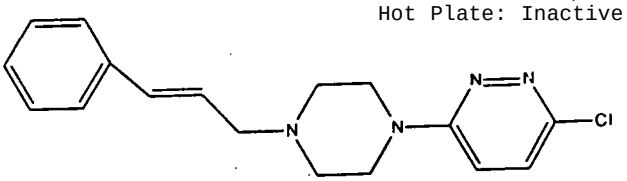
at a concentration of 10^{-7} M. It caused a parallel shift to the right (a 4.3-fold shift) in the sufentanil, concentration-effect curve. In the absence of NIH 10448, the EC50 for sufentanil was 3.47 nM ± 1.42 and the maximum response was a 100% inhibition of the twitch. In the presence of NIH 10448, the EC50 for sufentanil was 15.0 nM ± 10.4 and the maximum response was a 100% inhibition of the twitch.

SUMMARY

These are very unusual findings (i.e., the binding and vas deferens information are usually concordant) and the binding information was duplicated. The antagonist activity of NIH 10448 will have to be studied more extensively if the compound has interesting activity in the in vivo preparations. (See also NIH 10447.)

NIH 10496 (E)-3-Chlofo-6-[4-(3-phenyl-2-propenyl)-1-piperazinyl]pyridazine hydrochloride

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20



SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10496 were evaluated in five monkeys trained to respond and receive 0.32 mg/kg/inj codeine (Woods, 1980). Rates of responding maintained by doses of 0.01 and 0.03 mg/kg/inj NIH 10496 were nearly the same as rates maintained by saline. Rates of responding increased as doses of NIH 10496 increased above 0.03 mg/kg/inj, and reached a maximum at 1.0 mg/kg/inj. Higher doses could not be tested due to the limited water solubility of the drug. Rates of responding maintained by 1.0 mg/kg/inj NIH 10496 were nearly the same as rates of responding maintained by 0.32 mg/kg/injection codeine (Figure 1).

NIH 10496 (E)-3-Chloro-6-[4-(3-phenyl-2-propenyl)-1-piperazinyl]pyridazine hydrochloride

. . . (continued)

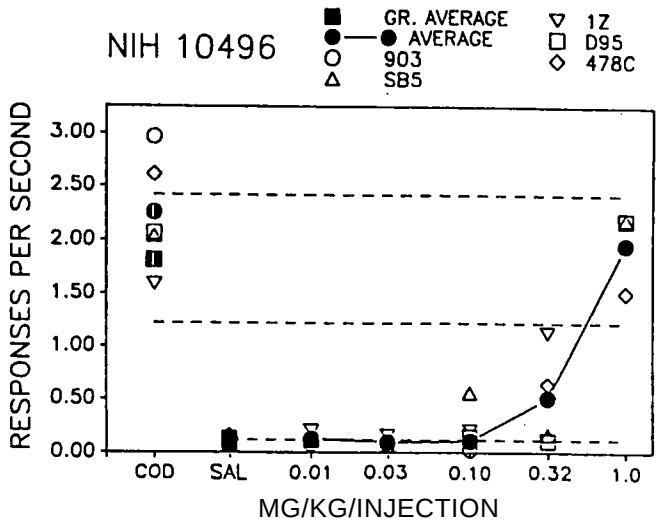


Figure 1. The data from individual monkeys are indicated by each animal's identification number and accompanying open symbol. The closed, connected circles indicate the average of the data from the monkeys in this experiment. Grand Average (closed squares) refers to a historical mean of 20 monkeys under the 0.32 mg/kg/injection codeine condition and the saline condition. The two topmost horizontal dashed lines indicate ± 3 SEM around the codeine grand average. The bottom dashed horizontal line indicates + 3 SEM of the saline grand average.

DRUG DISCRIMINATION IN RHESUS MONKEYS

NIH 10496 was studied in two rhesus monkeys discriminating between subcutaneous injections of 1.78 mg/kg of codeine and saline. In both monkeys studied, NIH 10496 substituted completely for the codeine discriminative stimulus (see Figure 2). For one subject doses of NIH 10496 larger than 0.1 mg/kg substituted for codeine and a second subject responded on the codeine lever only after a dose of 1.0 mg/kg NIH 10496. Rates of responding were decreased in a dose-related manner by increasing doses of NIH 10496 with responding eliminated at a cumulative dose of 3.2 mg/kg.

NIH 10496 (E)-3-Chloro-6-[4-(3-phenyl-2-propenyl)-1-piperazinyl]pyridazine hydrochloride

...(continued)

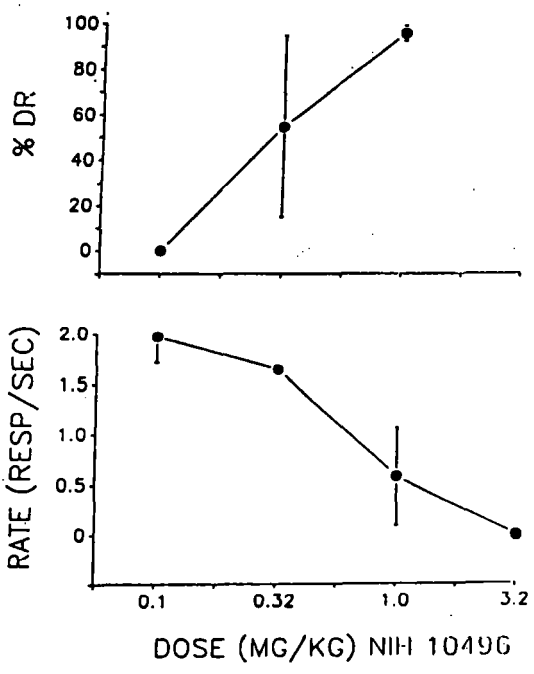
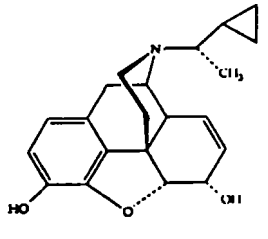


Figure 2

SUMMARY

NIH 10496 has significant morphine-activity in both behavioral assays.

NIH 10497 N-(1R-1-Cyclopropyl)ethylnormorphine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2.14 nM in presence of 150 mM NaCl.

NIH 10497 N-(1R-1-Cyclopropyl)ethylnormorphine hydrochloride

... (continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.72×10^{-8}	73.9%
After naltrexone	4.41×10^{-7}	79.6%
After ICI 174.864	5.39×10^{-8}	72.0%
After ß-FNA	4.20×10^{-8}	76.5%

NIH 10497 neither altered responses to sufentanil nor reversed sufentanil's inhibitory effects.

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10497 were evaluated in four rhesus monkeys trained to respond and receive 0.32 mg/kg/inj codeine (Woods, 1980). Rates maintained by NIH 10497 were generally above rates maintained by saline, but below rates maintained by codeine. The dose maintaining the highest rate of responding differed among subjects. See Figure 1.

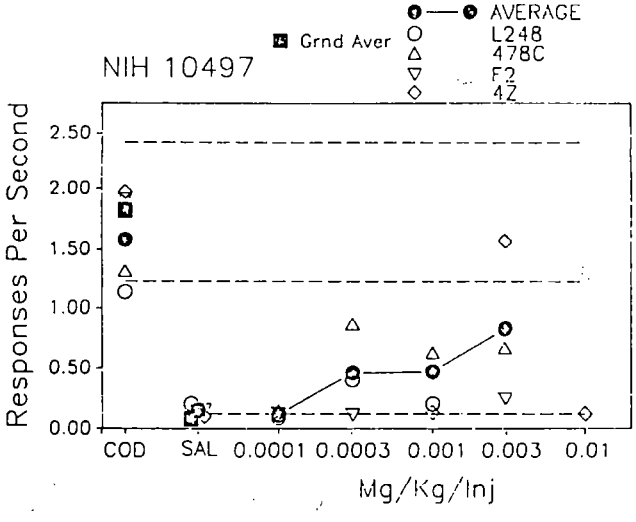


Figure 1. The data from individual monkeys are indicated in open symbols. The average data from these monkeys are indicated by

268-365 0 - 90 - 22 : QL 3

...(continued)

the closed and connected circles. Grand average refers to a historical control from 20 monkeys under codeine and saline conditions, and is shown by closed squares. The two topmost dashed horizontal lines indicate ± 3 SEM for the codeine grand average. The bottommost dashed horizontal line indicates + 3 SEM for the saline grand average.

DRUG DISCRIMINATION IN RHESUS MONKEYS

NIH 10497 was tested for discriminative stimulus effects in three monkeys trained to discriminate EKC from sham injections. NIH 10497 did not produce consistent EKC-like discriminative stimulus effects in any monkey, although one monkey responded completely on the EKC-appropriate lever at a single dose and returned to the sham-appropriate lever at the next higher dose. Response rates were suppressed at a dose of 0.10 mg/kg (See Figure 2).

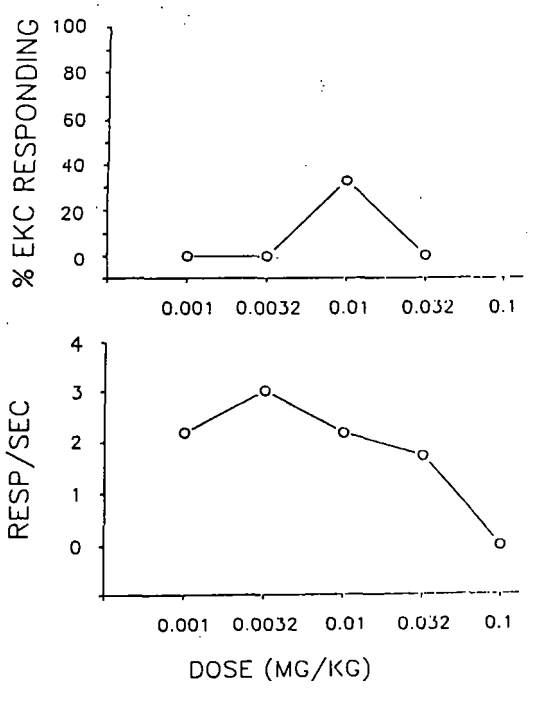


Figure 2

NIH 10497 was tested for discriminative stimulus effects in two monkeys trained to discriminate codeine from sham injections. One monkey generalized completely to codeine at a dose of 0.001

... (continued)

mg/kg NIH 10497 (Figure 3, closed circles). Response rates were suppressed at a dose of 0.032 mg/kg. Following administration of 0.10 mg/kg quadazocine, this monkey did not respond on the codeine-apppropriate lever until a dose of 0.32 mg/kg NIH 10497 (Figure 3, open circles). Response rates were normal at this dose following administration of quadazocine, despite the fact that they had been completely suppressed at this dose in the absence of quadazocine. The second monkey generalized completely to codeine at a dose of 0.01 mg/kg NIH 10497 (Figure 4).

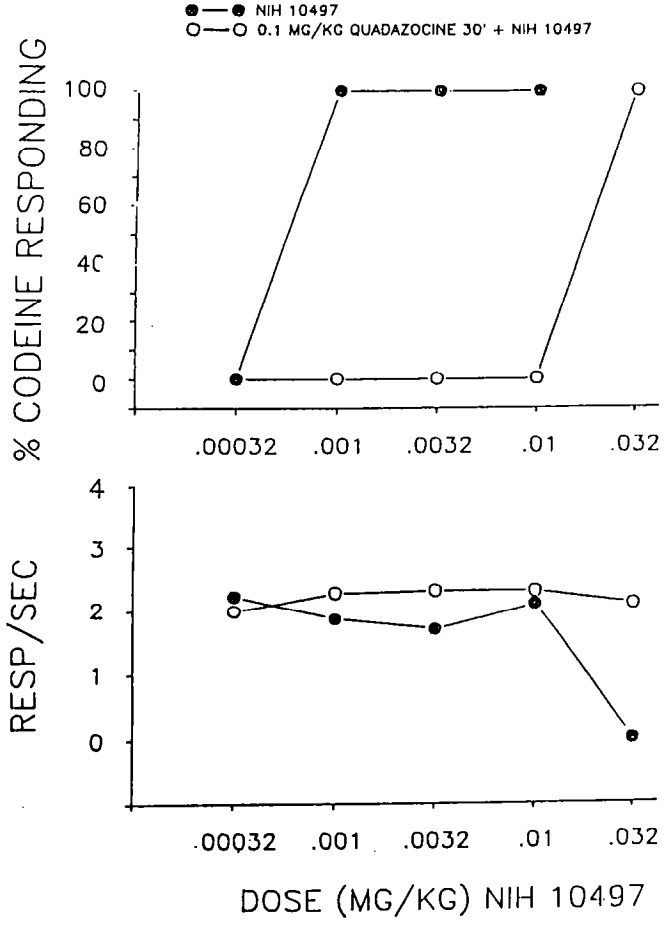


Figure 3

NIH 10497 N-(1R-1-Cyclopropyl)ethylnormorphine hydrochloride

...(continued)

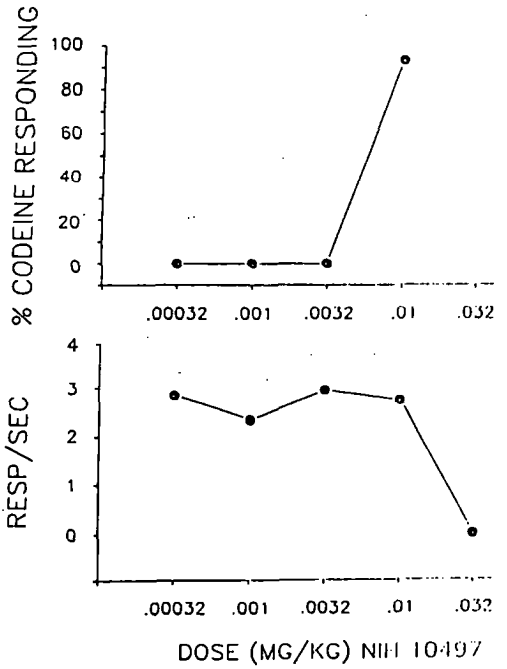
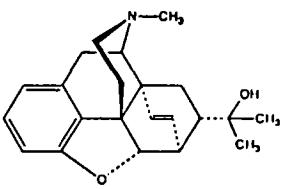


Figure 4

SUMMARY

NIH 10497 was a potent opioid in each of the preparations; it had a kappa agonist profile of activity in the mouse vas deferens. Its behavioral activities indicated a modest reinforcing effect and discriminative effects similar to codeine.

NIH 10501 4,5-α-Epoxy-α,α-N-trimethyl-6-,14-ethenoisomorphinan-7α-methanol



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 291 nM in presence of 150 mM NaCl.

NIH 10501 4,5-α-Epoxy-α,α-N-trimethyl-6-,14-ethenoisomorphinan-7α-methanol

...(continued)

MOUSE VAS DEFERENS PREPARATION

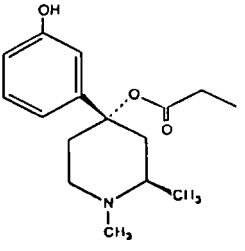
	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.81×10^{-7}	100%
After naltrexone	5.10×10^{-5}	100%
After ICI 174,864	5.92×10^{-6}	100%
After ß-FNA	2.85×10^{-5}	93.1%

NIH 10501 neither altered responses to sufentanil nor reversed sufentanil's inhibitory effects.

SUMMARY

NIH 10501 has delta-agonist actions in the vas deferens; its potency in both preparations was less than that of morphine.

NIH 10526 ß-1,2-Dimethyl-4-(3-hydroxyphenyl)-4-propionyloxy-piperidine hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive to 20

'DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2885 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

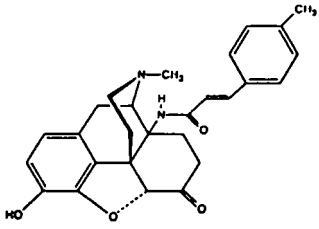
	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.02×10^{-8}	29.0%

NIH 10526 failed to block the actions of sufentanil. DSLET, or U50,488 in the smooth muscle preparation.

SUMMARY

NIH 10526 had very low potency in the binding assay and very low efficacy in the vas deferens preparation.

NIH 10544 14ß-(p-Methylcinnamoylamino)-7,8-dihydromorphinone mesylate



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive to 10

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING.

EC50 of 0.642 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

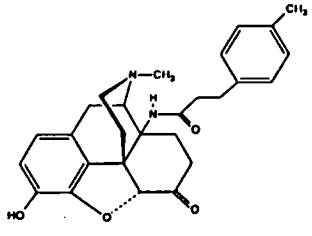
NIH 10544 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slooe
Sufentanil	8.55 ± 0.06	1.2
DSLET	7.77 ± 0.19	1.1
U50,488	7.39 ± 0.04	1.2

SUMMARY

NIH 10544 was a potent displacer of tritiated etorphine; it appears to be a relatively selective mu-receptor antagonist in the mouse vas deferens.

NIH 10545 14ß-(p-Methylphenylpropionylamino)-7,8-dihydromorphinone mesylate



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: 33% at 10

NIH 10545 14ß-(p-Methylphenylpropionylamino)-7,8-dihydromorphinone mesylate

... (continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.514 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	EC50 (M)	Maximum Response
Drug alone	6.59×10^{-9}	73.6%
After naltrexone	2.25×10^{-8}	62.3%
After ICI 174,864	1.13×10^{-8}	75.0%
After ß-FNA	1.24×10^{-8}	35.2%

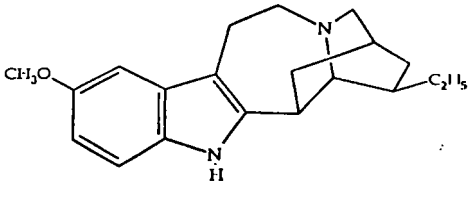
NIH 10545 neither altered responses to sufentanil nor reversed its inhibitory effects.

SUMMARY

NIH 10545 appears to be a more potent than and similar in efficacy to morphine; in the mouse vas deferens it may exert actions through mu and delta receptors.

NIH 10567 Ibogaine hydrochloride

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive to 20



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (24% inhibition at 6,000 nM) in presence of 150 mM NaCl.

NIH 10567 Ibogaine hydrochloride

. . . (continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.28×10^{-5}.	100%
With naltrexone	2.25×10^{-5}	100%

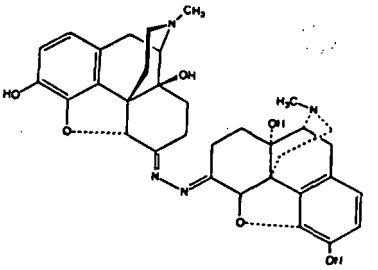
At a concentration of 10^{-6} M, NIH 10567 did not antagonize the actions of sufentanil, DSLET, or U50,488.

SUMMARY

NIH 10567 failed to demonstrate significant opioid activity in either in vitro preparation.

NIH 10571 Oxymorphazine dihydrochloride

MOUSE ANLGESIA, ED50, (mg/kg)
Hot Plate: 3.1 (2.2 - 4.3)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 45.1 nM in the presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	EC50 (M)	Maximum Response
Drug alone	5.77×10^{-7}	99.2%
After naltrexone	4.82×10^{-6}	100%
After ICI 174,864	4.12×10^{-7}	100%
After beta-FNA	1.52×10^{-6}	70.7%

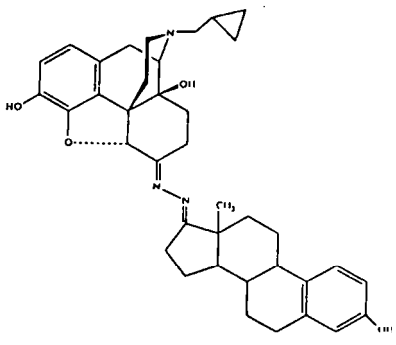
NIH 10571 Oxymorphazine dihydrochloride

... (continued)

SUMMARY

NIH 10571 had significant opioid activity in both preparations and the data were concordant with regard to potency estimates.

NIH 10572 Mixed azine of naltrexone and estrone



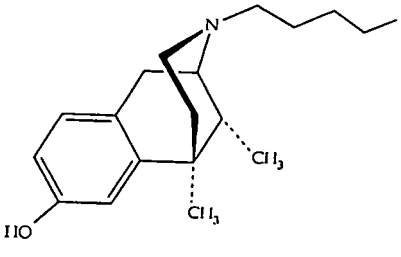
DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.65 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10572 was insolubie in 30% ethanol at a concentration of 3 x 10^{-4} M. It could not, therefore, be studied upon this preparation.

NIH 10573 (±)-5,9α-Dimethyl-2'-hydroxy-2-n-pentyl-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 173 nM in presence of 150 mM NaCl.

NIH 10573 (±)-5,9α-Dimethyl-2'-hydroxy-2-n-pentyl-6,7-benzomorphan hydrochloride

... (continued)

MOUSE VAS DEFERENS PREPARATION

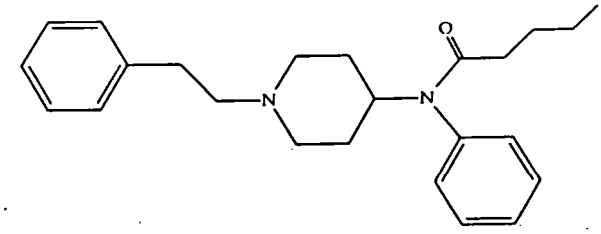
	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.08×10^{-6}	99.5%
After naltrexone	1.02×10^{-5}	93.0%
After ICI 174864	1.66×10^{-6}	98.7%
After ß-FNA	4.18×10^{-6}	94.0%

SUMMARY

NIH 10573 has mu-agonist actions in the vas deferens, and it displaces etorphine.

NIH 10576 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylvaleramide hydrochloride

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 7.5 (5.4 - 10.3)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 436 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10576 was studied upon the isolated, electrically stimulated mouse vas deferens preparation, in concentrations which ran ed from 10^{-9} M to 3×10^{-4} M. In concentrations of 10^{-8} M to 10^{-6} M this drug caused a very slight inhibition of the twitch (10 to 20%) which was blocked by naltrexone, 10^{-7} M, but not by ICI-174864, 10^{-7} M. NIH 10576 acted as an opioid antagonist. At concentrations from 10^{-8} M to 10^{-7} M this drug caused shifts to the right in the concentration-effect curves for sufentanil and at concentrations of 10^{-6} M and 10^{-5} M caused a marked decrease in the maximum response obtainable. The pA_2 value for this drug

NIH 10576 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylvaleramide hydrochloride

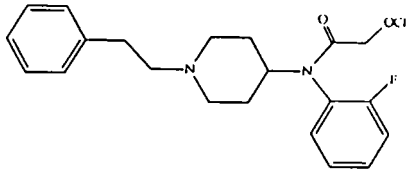
... (continued)

with sufentanil was 7.34 ± 1.13. The slope of the Schild plots suggests that the interaction might not be simply competitive. Concentrations of 10^{-5} M and 10^{-6} M NIH 10576 did not shift the DSLET concentration-effect curve. A higher concentration could not be tested against DSLET because the supply of 10576 has been depleted. The same concentrations of NIH 15076 also had no effect upon responses to U50,488 although a 10^{-5} M concentration caused a very slight shift to the right.

SUMMARY

NIH 10576 had opioid actions in both preparations. It was not very potent in the binding assay. It had interesting actions in the vas deferens, which suggests that it is a selective antagonist - if not specific - for mu-receptors.

NIH 10579 1-(2-Phenylethyl)-4-[N-(2-fluorophenyl)methoxyacetamido]piperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 49.2 nM in presence of 150 mM NaCl.

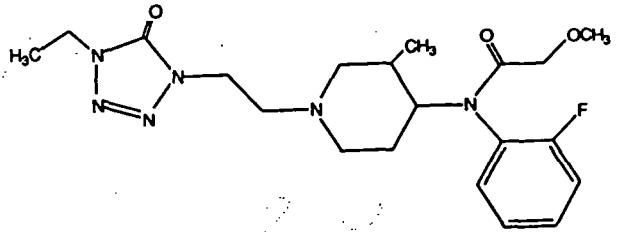
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.46×10^{-8}	99.6%
After naltrexone	7.25×10^{-7}	100%
After ICI 174864	3.61×10^{-8}	100%
After β-FNA	4.98×10^{-8}	77.5%

SUMMARY

NIH 10579 had significant opioid activity in both preparations. It was a mu-selective agonist in the vas deferens.

NIH 10580 1-[2-(4-Ethyl-4,5-dihydro-1H-tetrazolfn-5-one-1-yl)ethyl]3-methyl-4-[N-(2-fluorophenyl)methoxyacetamido]piperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 957 nM in the presence of NaCl.

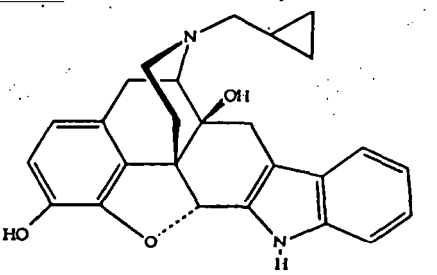
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.54×10^{-7}	87.0%
After naltrexone	9.47×10^{-6}	30.2%
After ICI 174864	4.86×10^{-7}	83.8%
After ß-FNA	2.02×10^{-6}	41.3%

SUMMARY

NIH 10580 had opioid actions in both preparations. In the vas deferens, it had mu-agonist actions.

NIH 10589 Naltrindole hydrochloride



BINDING IN MONKEY BRAIN CORTEX

EC50's were determined for the following receptor types: mu-9.53 nM, delta - 0.214 nM and kappa - 20.5 nM. These findings were obtained by displacing specific equilibrium binding of: 0.5 nM ^{3}H-sufentanil (mu-selective); 1.5 nM ^{3}H-DPDPE (delta-selective); and 1.5 nM ^{3}H-U69,593 (kappa-selective) in membranes from

NIH 10589 Naltrindole hydrochloride

... (continued)

monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.5) containing 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10589 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-6} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

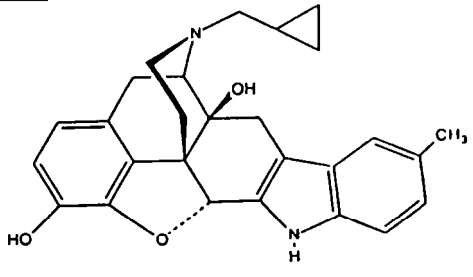
Agonist	pA_2 values	Slope
Sufentanil	7.71 ± 0.33	1.2
DSLET	9.44 ± 0.61	1.2

At a concentration of 10^{-7} M, NIH 10589 both slightly shifted the U-50,488 concentration-effect curve to the right and decreased the maximum responses to U50,488. Lower concentrations had no effect upon responses to U50,488.

SUMMARY

NIH 10589 in both preparations was a highly selective delta receptor ligand with selective antagonist activity in the vas deferens.

NIH 10590 Methyl naltrindole hydrochloride



BINDING IN MONKEY BRAIN CORTEX

EC50's were determined for the following receptor types: mu- 6.42 nM, delta - 0.204 nM and kappa - 9.01 nM. These findings were obtained by displacing specific equilibrium binding of: 0.5 nM ^{3}H-sufentanil mu-selective); 1.5 nM ^{3}H-DPDPE (delta-selective); and 1.5 nM ^{3}H-U69,593 (kappa-selective) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.5) containing 150 mM NaCl.

NIH 10590 Methyl naltrindole hydrochloride

...(continued)

MOUSE VAS DEFERENS PREPARATION

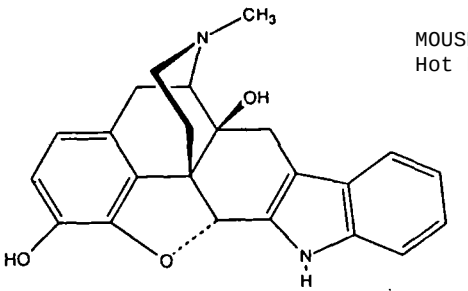
NIH 10590 studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	7.55 ± 0.51	1.5
DSLET	8.88 ± 0.89	1.6
U50,488	7.02 ± 0.52	1.2

SUMMARY

NIH 10590 is an opioid antagonist that is relatively selective for delta receptors. It is 21-fold more potent at delta than at mu receptors in mouse vas deferens. It is nearly 30-fold more potent than naltrexone at delta receptors and 1/16th as potent as naltrexone at mu receptors. It was also quite selective in the binding assay; having higher affinity for delta binding sites in brain.

NIH 10591 N-Methyl-N-nornaltrindole hydrochloride (Oxymorphindole)



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

BINDING IN MONKEY BRAIN CORTEX

EC50's were determined for the following receptor types: mu-1080 nM, delta - 3.37 nM and kappa - 688 nM. These findings were obtained by displacing specific equilibrium binding of: 0.5 nM ^{3}H-sufentanil (mu-selective); 1.5 nM ^{3}H-DPDPE (delta-selective); and 1.5 nM ^{3}H-U69,593 (kappa-selective) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer (pH 7.5) containing 150 mM NaCl.

NIH 10591 N-Methyl-N-nornaltrindole hydrochloride (Oxymorphindole)

... (continued)

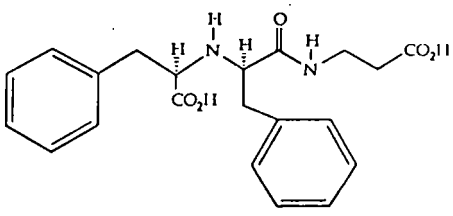
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.58×10^{-8}	61.2%
After naltrexone	5.44×10^{-7}	65.8%
After ICI 174864	1.17×10^{-7}	58.9%

SUMMARY

NIH 10591 exerted selective actions in both preparations. It had higher affinity for the delta binding site than others. It had opioid partial agonist actions in the vas deferens that were mediated through the delta receptor and, perhaps, through the mu-receptor as well.

NIH 10592 (S)-N-[1-Carboxy-2-phenyl)ethyl]-(S)-phenylalanyl-ß-alanine



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (1% inhibition at 6,000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	8.08×10^{-9}	30.3%
After ICI 174864	1.82×10^{-9}	42.7%

At a concentration of 10^{-6} M, NIH 10592 did not antagonize sufentanil, DSLET, or U-50,488. Naltrexone and ß-FNA completely blocked all responses to NIH 10592.

NIH 10592 (S)-N-[1-Carboxy-2-phenyl)ethyl]-(S)-phenylalanyl-ß-alanine

. . . (continued)

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10592 were evaluated in four monkeys experienced in responding and receiving intravenous infusions of 0.32 mg/kg codeine (Woods, 1980). The most notable factor evident in the pattern of self-administration of NIH 10592 was the marked variability both within and among monkeys. For Monkey 4Z, rates of responding were not maintained above those maintained by saline at any dose. For Monkey 8597, rates of responding maintained by NIH 10592 were above those maintained by saline, and below those maintained by codeine at all doses except the largest, but a typical inverted-U shaped dose-effect curve was not demonstrated by this monkey. Monkey 478C showed rates of responding close to those maintained by codeine at one dose only, a pattern that is more typical of drugs that are self-administered; this average rate (1.13 responses per second) is the average of one high rate (1.92 responses per second) and one fairly low rate (0.34 responses per second). A fourth monkey tested at this dose showed a similar pattern. This marked variability in response rates is very unusual. It may or may not be important to note that the highest rate of responding was always on the first presentation of a particular dose. The lower rate was on the second presentation, usually two days later. See Figure 1.

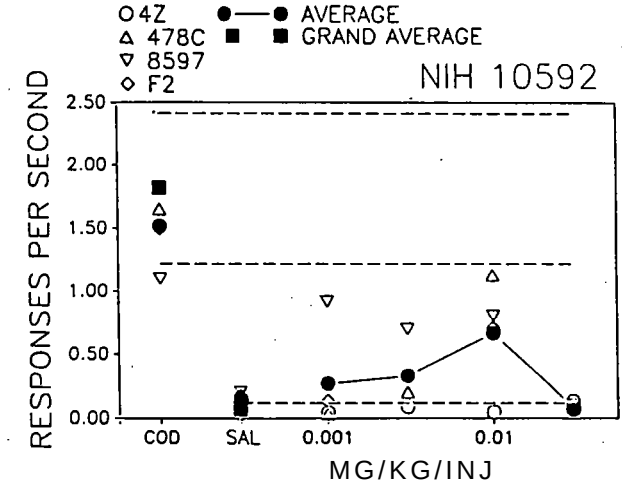


Figure 1. The data from individual monkeys are indicated by each animal's identification number and the accompanying open symbols (an average of two observations at each point). The closed circles are averages of these individual data. The closed squares at the COD and SAL points are historical averages of data

NIH 10592 (S)-N-[1-Carboxy-2-phenyl)ethyl]-(S)-phenylalanyl-ß-alanine

... (continued)

obtained in a group of 20 monkeys after conditions of 0.32 mg/kg/inj codeine and of saline self-administration. The topmost dashed lines are - 3 standard errors of the mean of the codeine grand average; the bottommost dashed line is + 3 standard errors of the mean of the saline grand average.

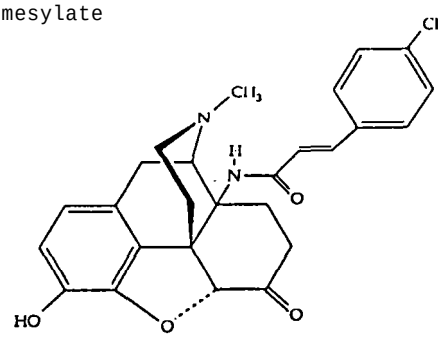
DRUG DISCRIMINATION IN RHESUS MONKEYS

NIH 10592 was studied in two rhesus monkeys discriminating between s.c. injections of 1.78 mg/kg of codeine and saline. Up to the largest dose, because of solubility limits, that could be studied (17.8 mg/kg), NIH 10592 had no effect on response rates and occasioned responding only on the saline lever.

SUMMARY

NIH 10592 was inactive in the binding and drug discrimination assays under the conditions studied. It was active in the mouse vas deferens as a mu-agonist with low apparent efficacy. It produced some evidence of reinforcing effect; the data were unusually variable. This is an unusual set of findings and should be examined in more detail when more is known about the compound in the other assays.

NIH 10594 14ß-(p-Chlorocinnamoylamino)-7,8-dihydromorphinone mesylate



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.301 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10594 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

HIH 10594 14ß-(p-Chlorocinnamoylamino)-7,8-dihydromorphinone mesylate

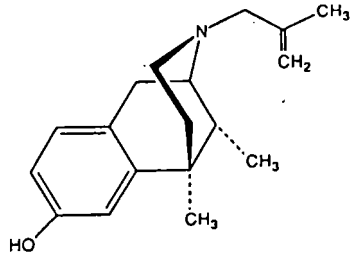
... (continued)

Agonist	pA_2 values	Slope
Sufentanil	9.83 ± 0.37	1.0
DSLET	8.78 ± 0.71	1.4
u50,488	8.24 ± 0.57	1.6

SUMMARY

NIH 10594 was quite potent in both preparations. Its antagonist action in the vas deferens suggests selectivity at the mu receptor. This will be followed up in the binding assays in monkey brain with selective ligands.

NIH 10595 (-)-5,9α-dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 77.6 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.73×10^{-7}	51.4%
After naltrexone	7.71×10^{-7}	57.5%
After ICI-174864	6.14×10^{-7}	36.6%

NIH 10595 acted also as an opioid antagonist. pA_2 values against the following agonists were as follows:

Agonist	pA_2 values	Slopes
Sufentanil	6.77 ± 0.39	1.1
DSLET	6.28 ± 0.28	0.8
U50,488	7.06 ± 0.40	1.1

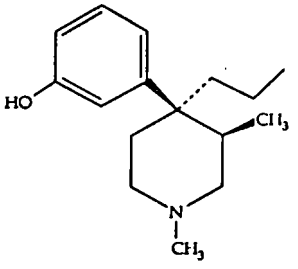
NIH 10595 (-)-5,9α-dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride

. . . (continued)

SUMMARY

NIH 10595 is an opioid mixed agonist-antagonist less potent than naltrexone at mu, delta, and kappa receptors. The agonist activity of this drug appears to be upon delta receptors.

NIH 10597. 4-(3-Hydroxyphenyl)-1,3-dimethyl-4-n-propylpiperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1026 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

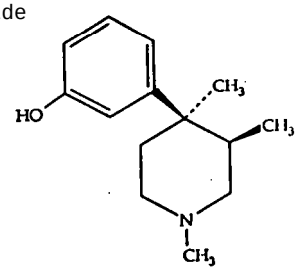
NIH 10597 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.14 ± 0.42	1.0
DSLET	6.47 ± 0.49	1.1
U50,488	< 5.52	

SUMMARY

NIH 10597 had low potency in each preparation; the pattern of antagonist activity in the vas deferens suggests that it is slightly selective for the mu and delta opioid receptors.

NIH 10598 4-(3-Hydroxyphenyl)-1,3,4-trimethylpiperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 403 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

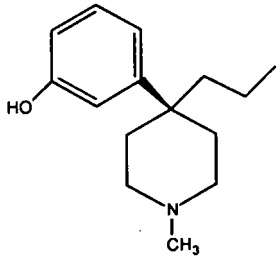
NIH 10598 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.91 ± 0.46	1.1
DSLET	5.92 ± 0.29	0.7
U50,488	6.43 ± 0.36	1.1

SUMMARY

NIH 10598 had a low potency in the binding assay as well as the vas deferens. Its antagonistic characteristics in the vas deferens suggest a slightly higher affinity for mu and kappa receptors than delta. This suggestion will be assessed with binding assays in monkey brain with selective ligands.

NIH 10599 4-(3-Hydroxyphenyl)-4-n-propyl-1-methylpiperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 146 nM in presence of NaCl.

NIH 10599 4-(3-Hydroxyphenyl)-4-n-propyl-l-methylpiperidine hydrochloride

... (continued)

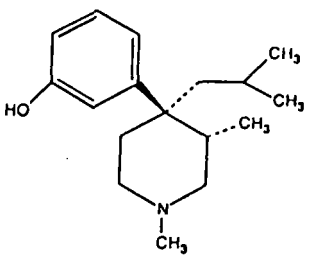
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.58×10^{-7}	90.9%
After naltrexone	1.95×10^{-6}	35.8%
After ICI-174864	5.53×10^{-7}	92.4%
After ß-FNA	1.12×10^{-6}	33.9

SUMMARY

NIH 10599 had mu-agonist actions in the vas deferens; its potency in the binding assay agreed with that in the vas deferens.

NIH 10600 4-(3-Hydroxyphenyl)-1,3-dimethyl-4-(2-methyl-prop-1-yl)piperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 46.3 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

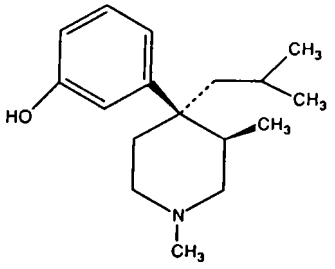
	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.31×10^{-7}	31.9%

Both naltrexone and ICI 174,864 completely blocked the inhibitory actions of NIH 10600. NIH 10600 did not antagonize responses to sufentanil, DSLET or U-50,488.

SUMMARY

This is a curious compound due to the disparity of results between the two preparations. It has opioid activity at lower concentrations than morphine in the binding assay, but lacks high efficacy or antagonist activity in the vas deferens.

NIH 10601 4-(3-Hydroxyphenyl)-1,3-dimethyl-4-(2-methyl-prop-1-yl)piperidine hydrochloride.



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1743 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

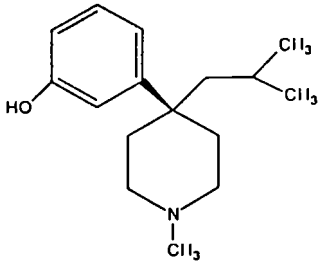
NIH 10601 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens and it was evaluated as en antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	5.63 ± 0.52	1.5
DSLET	6.23 ± 0.39	1 . 2
U50,488	5.66 ± 0.44	1.3

SUMMARY

NIH 10601 had low opioid potency in both preparations. Its antagonist profile suggests activity at each of the opioid receptors in the vas deferens.

NIH 10602 4-(3-Hydroxyphenyl)4-isobutyl-1-methylpiperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 76.1 nM in the presence of NaCl.

NIH 10602 4-(3-Hydroxyphenyl)4-isobutyl-1-methylpiperidine hydrochloride

... (continued)

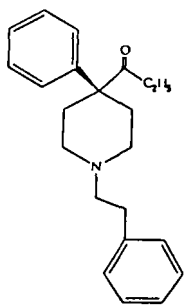
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.59×10^{-7}	96.0%
After naltrexone	4.75×10^{-6}	53.4%
After ICI-174864	2.12×10^{-7}	93.7%
After ß-FNA	1.71×10^{-7}	97.0%

SUMMARY

NIH 10602 had concordant potencies in the two preparations and was a mu agonist in the vas deferens preparation.

NIH 10603 N-Phenylethyl-4-phenyl-4-propionylpiperidine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1399 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10603 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3×10^{-4} M. This drug did not inhibit the twitch at any. concentration and was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	7.09 ± 0.58	1.6
DSLET	6.39 ± 0.34	1.0
U50,488	5.94 ± 0.31	0.9

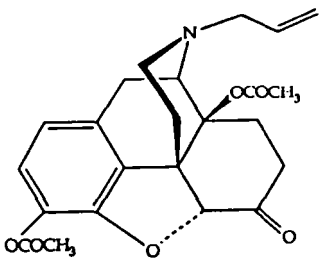
NIH 10603 N-Phenylethyl-4-phenyl-4-propionylpiperidine hydrochloride

...(continued)

SUMMARY

NIH 10603 is an opioid antagonist that is less potent than naltrexone at mu, delta, and kappa receptors in the vas deferens. The binding date are consistent with its low potency in the vas deferens.

NIH 10605 Naloxone 3,14-diacetate



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 11.8 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

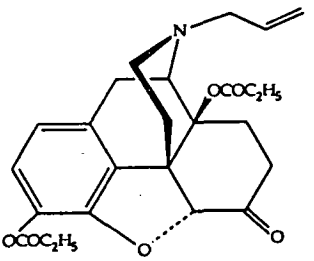
NIH 10605 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration and was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slopes
Sufentanil	8.03 ± 0.16	1.23
DSLET	7.65 ± 1.16	1.41
U50,488	7.39 ± 1.01	1.21

SUMMARY

NIH 10605 is an opioid antagonist which is less potent than naltrexone at mu, delta, and kappa receptors. Note that the slopes of the Schild plots are considerably greater than unity. It is also less potent than naltrexone in the binding assay.

NIH 10606 Naloxone 3,14-dipropionate maleate



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 59.2 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10606 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration and was evaluated as an antagonist. pA_2 values against the following agonists were:

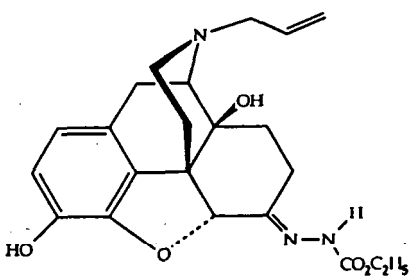
Agonist	pA_2 values	Slopes
Sufentanil	8.43 ± 0.28	0.8
DSLET	7.25 ± 0.46	1.3

NIH 10606 was a non-competitive antagonist of U-50,488. Thus, a pA_2 value could not be determined.

SUMMARY

NIH 10606 was a selective mu-receptor antagonist in the vas deferens; slightly less potent than naltrexone. It was also less potent in the binding assay.

NIH 10607 Naloxone ethoxycarbonylhydrazone



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 7.20 nM in the presence of NaCl.

NIH 10607 Naloxone ethoxycarbonylhydrazone

. . . (continued)

MOUSE VAS DEEERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
'Drug alone	1.02×10^{-6}	32.7%
After naltrexone	3.47×10^{-6}	26.6%
After ICI 174864	6.14×10^{-7}	36.6%

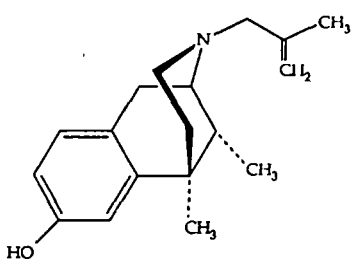
NIH 10607 also acted as an opioid antagonist. pA_2 values against the following against were as follows:

Agonist	pA_2 values	Slopes
Sufentenil	7.93 ± 0.30	0.9
DSLET	7.38 ± 0.30	0.9
U50,488	7.33 ± 0.43	1.1

SUMMARY

NIH 10607 is a partial opioid agonist which has significant antagonistic activity. As an antagonist, it is less potent than naltrexone at mu, delta, and kappa receptors. It is less potent than naltrexone in the binding assay, as well.

NIH 10608 (+)-5,9α-Dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (23.4% inhibition at 6,000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10608 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M *to* 3 x 10^{-5} M. *No concentration* of this drug inhibited the twitch of the vas deferens. In a concentration of 10^{-6} M, this drug failed to antagonize the inhibitory actions of sufentanil, DSLET, or U50,488.

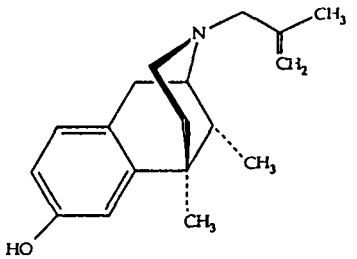
NIH 10608 (+)-5,9α-Dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride

. . . (continued)

SUMMARY

NIH 10608 failed to display significant opioid activity in either in vitro preparation.

NIH 10612 (+)-5,9α-Dimethyl-2'-hydroxy-2-(2-methylpropenyl)-6,7-benzomorphan hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 143 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

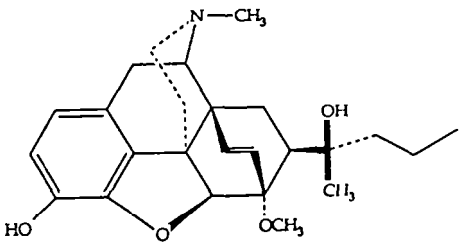
NIH 10612 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration and was evaluated as an antagonist. pA_2 values against the following agonists were:

Agonist	pA_2 values	Slope
Sufentanil	6.82 ± 0.39	1.1
DSLET	5.97 ± 0.31	0.9
U50,488	6.76 ± 0.36	1.1

SUMMARY

NIH 10612 is an opioid antagonist that is less potent than naltrexone at the mu, delta, and kappa receptors. It was also much less potent than naltrexone in the binding assay.

NIH 10617 (+)-Etorphine hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (10% inhibition at 6,000 nM) in the presence of NaCl.

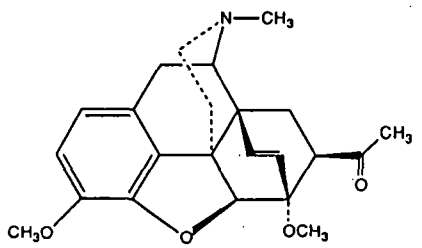
MOUSE VAS DEFERENS PREPARATION

NIH 10617 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration. At a concentration of 10^{-5} M, this drug did not shift the concentration effect curves for sufentanil, DSLET, and U50,488.

SUMMARY

NIH 10617 had no significant opioid activity in these preparations.

NIH 10620 (+)-Thevinone hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6,000 nM (1% inhibition at 6,000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10620 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration. NIH 10620, up to 10^{-5} M, did not shift appreciably the concentration-effect curves for sufentanil, DSLET, or U50,488.

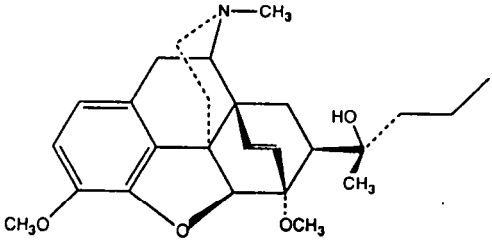
NIH 10620 (+)-Thevinone hydrochloride

. . . (continued)

SUMMARY

NIH 10620 had significant opioid antagonist activity in the mouse vas deferens without having such affinity for the central receptor.

NIH 10621 (+)-19-Propylthevinol oxidate



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of >6,000 nM (2.7% inhibition at 6,000 nM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10621 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. This drug did not inhibit the twitch at any concentration. NIH 10621, up to 10^{-5} M, did not shift appreciably the concentration-effect curves for sufentanil, DSLET, or U50,488.

SUMMARY

NIH 10621 failed to display significant opioid activity in these *in vitro* preparations.

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Woods, J.H. Narcotic-reinforced responding: A rapid evaluation procedure. Drugs and Alcohol Dependence 5:223-230, 1980.

AFFILIATION

The Drug Abuse Basic Research Program, Departments of Pharmacology and Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-0626

ERRATUM

The EC50 of NIH 9835 was stated to be 0.0187 nM, in:

Woods, J. H., Winger, G. D., Medzihradsky, F., Smith, C. B., Gmerek, D., Aceto, M.D., Harris, L. S., May, E. L., Balster, R. L., Slifer, B. L.: Evaluation of New Compounds for Opioid Activity in Rhesus Monkey, Rat, and Mouse (1984). In: Harris, L. S. ed. Problems of Drug Dependence: 1984. National Institute on Drug Abuse Research Monograph. Washington, D. C.: supt. of Docs., U. S. Govt. Print. Off., 1985, p. 336.

The EC50 should be 1.7nM.

Subject Index

In order to simplify the Index, the subject subheadings along with page numbers can be found under both the chemical name and the NIH number.

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DHHS Publication No. (ADM)90-1663
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