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The College on Problems of Drug Dependence, Inc.

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Problems of Drug Dependence, 1993:

Proceedings of the 55th Annual Scientific Meeting, The College on Problems of Drug Dependence, Inc.

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Louis S. Harris, Ph.D.

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This volume of the Proceedings of the College on Drug Dependence, 1993 is dedicated to the memory of Drs. William R. Martin and Daniel X. Freedman, two distinguished scientists whose outstanding contributions to drug abuse research, treatment, and public policy are recognized nationally and internationally. Their dedication over many years to the College on Problems of Drug Dependence deserves particular acknowledgement. We will sorely miss them.

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IN MEMORIAM: WILLIAM ROBERT MARTIN, M.D.
1921-1993

On May 27, Dr. William R. Martin died in a tractor accident at his home in Midway, Kentucky.

It is fitting that we pay tribute to Bill at the CPDD, as this is an organization with which he was closely affiliated for more than 30 years. He presented frequently at these meetings, served on the Board of Directors, received the Nathan B. Eddy Award, and was a Charter Fellow of the College.

Dr. Martin is probably best known to this audience from his years at the Addiction Research Center (ARC) in Lexington, Kentucky and then at the Universitv of Kentucky. Bill was born in 'Aberdeen. South Dakota, 72 years ago. After earning his undergraduate degree at the University of Chicago, he earned his M. D. and M. S. in Pharmacology at the University of Illinois. An internship at the Cook County Hospital and a faculty position at Illinois in Pharmacology preceded his arrival at the ARC in 1957 as a staff scientist. He became Director of ARC, taking over from Harris Isbell in July of 1963 (coincidentally on the day 1 arrived as a young medical officer fresh out of internship).

Don Jasinski joined the ARC staff two years later. Don and I were but two of the many students and young scientists who were privileged to have Bill Martin as their primary mentor. Bill retired from the Public Health Service (PHS) and the ARC in 1977 and went across town to become the Chairman of Pharmacology at the University of Kentucky Medical Center, a position he held until 1990. Never one to stop working, he then became a Research Professor in the Department of Anesthesiology, where he continued his research in close collaboration with Dr. Jewell Sloan, his student and colleague for more than 35 years.

Bill Martin's work in substance abuse is well known to this audience, from his work in multiple opioid receptors (begun in the 60s), to studies in sedative hypnotics, stimulants and hallucinogens, to his more recent work on nicotine receptors. His work and interests spanned a broad range from fundamental preclinical research to experimental clinical

studies, to research in treatment modalities, to concerns with drug abuse in the broadest social, medical and geo-political contexts. Bill's ideas and writings have helped to shape the directions of our field for the past two decades and will likely remain influential for several more. The ideas he proposed and the body of work he produced is an important legacy.

Over the 20 years Bill spent at the ARC and 15 more at the University of Kentucky, several generations of young scientists, graduate. students and medical students came under the influence of his teachings. We learned much from him - not only substantive knowledge in pharmacology and substance abuse, but also principles of intellectual integrity and a commitment to the continued striving for excellence and the search for new knowledge. We, his students and colleagues, am his second legacy, perhaps in some ways even more enduring and far reaching than his first, as we pass his lessons on to further generations of students and young colleagues.

Although we tend to key on Bill Martin, the Scientist, let us also pause a moment to remember Bill Martin, the Man. Bill was a true gentleman and egalitarian. I spent more than ten years as his EEO Officer at the ARC and learned well the depth of his feelings for fairness and equality. He was also a deeply caring and compassionate man.

We send our heartfelt condolences to Bill's wife, Catherine, and his children, Kathy, David and Douglas. And we thank Bill for the lessons he taught us, for the ideals and principles he helped us shape into our own systems of values, for his mentorship and for his friendship. We will miss him.

Charles W. Gorodetzky

IN MEMORIAM: DANIEL X. FREEDMAN, M.D.
1921 - 1993

Daniel X. Freedman, the Louis Block Professor Emeritus of Psychiatry at the University of Chicago, and since 1983, the Judson Braun Professor of Psychiatry and Pharmacology at UCLA, died in his sleep at his home in Los Angeles on June 2, 1993, at the age of 71, He is survived by his wife, Mary.

The contributions of Danny, as he was known to his friends, to the development of a scientific foundation for the practice of psychiatry arc inestimable. Trained as a physician, psychiatrist, psychoanalyst, and pharmacologist, he had an extremely productive and influential career as a scientist, teacher, writer, editor, policymaker and practitioner.

Danny received his undergraduate degree from Harvard University and then went to Yale where he completed medical school and a residency in psychiatry. He joined the faculty at Yale in 1956 and remained there until 1966. During that period Danny began the pursuit of his life long goal of understanding the neurochemical basis of the actions of LSD and other hallucinogenic drugs. In addition, working with colleagues and students, Danny found that environmental stress could alter the levels and effects of the brain neurotransmitters serotonin and norepinephrine. Further, he discovered the presence of high levels of serotonin in the pineal gland of the brain and that severely retarded and autistic children had abnormally high levels of brain serotonin. Perhaps even more important than Danny's own research was his establishment of an NIMH training program in which basic biological scientists and clinicians worked side by side to solve the mysteries of the biological basis of mental disorders. During the period at Yale, Danny became a leader in the revolution to demonstrate that a scientific base for the applications of psychiatry was possible.

In 1966, Danny became the chairman of the Department of Psychiatry at the University of Chicago where he continued his activities as a researcher, teacher, editor of the Archives of General Psychiatry, mentor to his residents and junior staff and a major force in shaping policy issues in the area of mental health at both the state and national level.

He kept the airlines in business shuttling back and forth from Chicago to Washington to serve on innumerable government committees. When he wasn't in Washington, he was in constant phone contact with policymakers, dispensing his sage advice in his own inimitable style. At the University of Chicago, Danny also built one of the most unique and respected departments of psychiatry by continuing to foster the interaction of basic research scientists with clinicians. It was during this period of time that I was recruited by Danny to join the Department of Psychiatry. For 15 years I had the delightful opportunity to work with Danny and observe his interactions with students and colleagues. His energy and enthusiasm for brain and behavioral research were boundless and infectious. Coupled with his sensitivity and concern for the victims of mental and addictive disorders, he was an exemplary role model and teacher for all of us.

Danny was also a very romantic person which was obvious to all that saw him with his wife Mary. His romantic sense was brought home to me personally when my youngest daughter was born and came home from the hospital. We received a package from the local florist and laughed at what we believed was their mistake when we saw that it was a corsage. The message however set us straight. It said: "To Alyson-I wanted to be the first man in your life to send you flowers-Love, Danny Freedman".

While I served as the Director of NIDA, I was always comforted by the fact that when I was really in a quandary I could call Danny Freedman night or day and spend as long as necessary to get his advice, solace and support I know that he served this same function for innumerable people. We will all miss him terribly, but take heart in the fact that his influence, through his writings and the students and colleagues who were fortunate enough to learn from him, will remain.

Charles R. Schuster

ANY MAN'S DEATH: PRESIDENTIAL ADDRESS

T. J. Crowley

I am honored to have been associated with the Board of Directors of the College on Problems of Drug Dependence since 1988, and to have served as President of CPDD during the last year, as it became a membership society. I feel honored because it is the members of this College who have taught me what I know. It is now nearly a third of a century since I finished medical school, and in my professional lifetime the field has moved incredibly. Unfortunately, as our knowledge grew so did our frustrations, and I want to talk about both.

First let me remind you of a dozen of those advances. This is a personal list; others will make other lists, but these are the changes that have affected my work. A good benchmark of where a field stands is what it teaches its students. As a junior medical student I was assigned as a psychiatry text Noyes and Kolb (1958). In it we were told that "drug addiction is usually symptomatic of a personality disorder". Addicts were described in these terms: emotionally immature, hostile, aggressive, seeking relief from inner tension, with immature drives for immediate goals, and frankly neurotic with anxiety and obsessive compulsive symptoms. It was stated that relapse is "actually but a rationalization of their emotional defects". We were told that the patient "usually has a resistance to gaining insight into the real causes of his addiction", as though the authors of the book were quite sure of such causes. "The moral deterioration in the cocaine addict is even greater than in the morphine habitue", they said. And after this intensely negative description of addiction, we were told that therapists "will not be critical or judging".

Behind such pseudo-scientific jargon was a blaming and reviling of addicted persons, and while we may now laugh at such material, an entire generation of American physicians left medical school believing that that stuff summed up the addictions. It is amazing that any of us came to work in this field.

But since then ...

1) Scientists in our field have laid to rest much of that psychologizing with concrete evidence that animals self-administer most of the same drugs that people take.

2) Members of this organization have used learning theory to make sense out of the complex behaviors of drug dependent persons.

3) Our members have shown that dependence on alcohol and nicotine are problems of drug dependence, and those are propositions with profound public health impact.

4) Scientists here with us today have demonstrated that definable receptors, transmitters, and pathways mediate in central nervous system the process of drug reinforcement.
5) Our colleagues have proved a genetic vulnerability to certain kinds of drug dependence, and have organized data strongly suggesting a genetic influence in most drug dependence.
6) Epidemiologists belonging to CPDD have shown that substance abuse and dependence diagnoses comprise 3 of the 4 most prevalent psychiatric disorders in the U.S. general population.
7) Those same investigators have demonstrated that other psychiatric disorders, especially Antisocial Personality Disorder, very frequently have associated substance abuse or dependence.
8) Sadly, we now have recognized that nearly one-fourth of the 2 million U.S. deaths each year are premature deaths due to drug abuse or dependence (Figure 1).

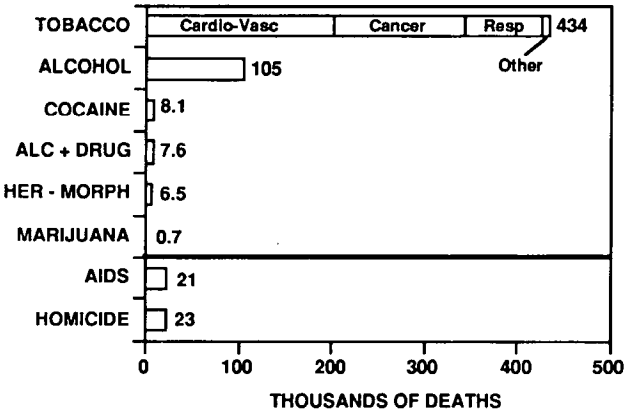

FIGURE 1. Estimated Annual Mortality, Drug and Related Problems (about 1990). Tobacco estimates from Shultz (1991). Alcohol estimates from Schultz et al (1990). Other-drug estimates: (all US deaths from *US Vital Statistics)* X [deaths involving various drugs in all DAWN jurisdictions (DAWN 1990) / all deaths in all DAWN jurisdictions *(US Vital Statistics)].* AIDS, Homicide from US *Vital Statistics.* "Alc + Drug", alcohol in combination with another drug; "Her - Morph", heroin or morphine.

9) Giants in the history of CPDD proved that agonist and antagonist drugs could be cornerstones of successful treatments for opioid dependence.
10) We have learned that contingency management, the arrangement of

rewards and punishments, powerfully could suppress drug use.
11) Fellows of CPDD found that psychotherapy combined with methadone notably improves outcome in treatment.
12) And our members have demonstrated efficacy of HIV prevention activities among addicts.

And so, in my professional lifetime our field has stopped simply blaming and despising addicts. We have applied the best biological, clinical, and social science techniques to these problems. We have made extraordinary advances in understanding etiology. Our research has illuminated social policy and focused prevention, has improved therapies, and it *has made people's lives better.*

But sometimes the frustrations are so great that it is hard to keep going. Let me add a baker's dozen of those frustrations.

It is *draining* for us to deal with lawmakers who seem unable to understand public health problems. Some years ago I and some colleagues testified on funding for substance treatment programs to the Colorado legislature. When we were done a member of that legislative committee pounded on the table and said to us, "We have been funding these alcoholism programs for 10 years, and you guys still haven't solved this problem!"

It is *draining* for us to deal with hospital administrators and other clinicians who ignore the mortality data, who ignore what really is killing our fellow citizens, and who make it clear that they do not want addicts defiling their Temples of Health. Emphysema patients are dying of nicotine addiction, and just last year our federally-funded Clinical Research Center disapproved a study in their Ambulatory Care Area of rapid smoking therapy for emphysema patients. That therapy, which involves having addicts rapidly smoke a number of cigarettes, was rejected, the Center reviewers said, because we now have a non-smoking hospital!

It is *draining* for us to deal with university administrators who do not want any bad press generated from the support of drug-related animal research.

It is *draining* for us to deal with hostile Animal Research Committees. Ours once reviewed an application of mine and concluded that developing drug self-administration in animals "may be considered inhumane", while literally millions of our fellow citizens, *all humans,* are addicted to crack, heroin, alcohol, nicotine, and numerous other drugs.

It *drains* us to fend off animal-rightists' insults. These people think they indict us when they taunt us about a willingness to kill monkeys to save junkies. And they tell our fellow Americans that BIGGEST LIE, that nothing useful has come from animal research in our field, when in fact the discovery of self-administration of drugs among animals produced a sea-change in the field, and

when nearly all of our understanding of the CNS basis of drug abuse has come from animal research. They ignore the nearly half-million drug-related deaths annually, instead writing letters to local newspapers -- as they have about me -- decrying "the horrors of the lab". And this was despite the fact that my colleagues and I ran a huge, beautiful outdoor monkey-park, where the animals had trees, grass, and lovely views.

It is *draining* for us to deal with university officials who appoint, as they did in Colorado, an animal rights activist to our Institutional Animal Care and Use Committee, saying "Now we can work with them". That guy then voted against every protocol presented to the committee. Moreover, he collected those protocols and later published many of them in an animal-rights journal.

It *drains* us constantly to be on guard against break-ins and sabotage by animal terrorists.

About half mile from where my monkey lab stood, my colleagues and I have a residential treatment program for deeply troubled, drug-dependent adolescents. American society could afford to send squadrons of veterinarians frequently to inspect that gorgeous monkey facility to assure that we never mistreated a monkey. But society apparently could not afford enough child protection workers to intervene in the squalid neglect, the violent physical abuse, and the revolting sexual abuse that characterize the history of almost every youth admitted to our treatment program, *and it drains* us to have such priorities forced on our field.

It *drains* us to deal with loonies of assorted stripes who ignore the clear empirical data and still tell us, most recently on 60 Minutes national television, that methadone maintenance treatment is either ineffective or immoral.

It is *draining* for us to work with fellow professionals who believe that drug abuse is just too dirty to be a subject of science. When the cocaine epidemic began we knew almost nothing about cocaine use, and I sent a grant application to the National Institute on Drug Abuse asking to go into the community to watch people use cocaine, an ethnographic study. But my own Human Subjects Committee refused approval saying that such work was inappropriate for a University Professor. I eventually withdrew the application after a pediatrician on that committee said, "You want to study drug abuse. Well, I study child abuse, but I don't go into people's homes to watch them beat up their kids!"

Tens of thousands of Americans now use cocaine in back-alleys every day. It is *draining* for us to deal with well-intended but misguided people who say (safety questions) that it is morally unconscionable to study this scourge by observing the cocaine use of a few of those people in our laboratories.

It is *draining* for us to deal with a despising public, represented by that woman

who sat next to me on an airplane last year. She asked what I do, and perhaps unwisely, I told her. She listened for a moment or two and then said, almost sneering, "You try to treat people who act like that?".

And yes, the patients whom we study and treat *drain* us. They often do not accept or respond to the best treatments that we can offer. They sometimes continue to take terrible risks with their lives. They frequently seem to care less about themselves than we care about them. Some commit suicide; some commit homicide.

So we who would understand drug-taking are ringed about by the friends of darkness. On the one hand are those who say that we cannot go to the places where addicts use drugs, for that is beneath us. On the other hand are those who would not permit us to examine drug taking by humans in our hospitals and laboratories. Out ahead are the animal-rights crazies, who revile us for studying drug use in animals. And always, always behind us are the sounds of thousands, and thousands, and thousands of dying people.

So why do we continue? We are all smart people who could make more money in real estate.

One reason that we continue is that we know of the suffering caused by these disorders, and *we* are *committed to make some* difference.

And I think there also is a shared experience which keeps us going. It is the intense support we give each other in this meeting, as we do our best to address the great public health problem of our time. Oh sure, we fight and argue and disagree about the data, but even in that we support each other in a joint search for a truth that may make people's lives better.

I think that what keeps us all going was summed up almost 400 years ago by the clergyman and poet, John Donne. You know the lines; you may know the story. Donne had officiated for many years at funerals, and then fell ill himself. But from his rectory sick bed he still could hear the funeral bells. We see Figure 1 when reading what he wrote then ...

> Any Mans *death* diminishes *me,* because I am involved in *Mankinde;* and therefore never send to know for whom the *bell* tolls; It tolls for *thee.*

Friends, colleagues, there is a simple moral to this lament. We can use CPDD to support one another. If you think that you qualify for membership in this organization, but you haven't yet joined, do. In this field, as in no other, we desperately need each other's support. And thank you for making me your President this year.

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DRUG ABUSE RESEARCH ACCOMPLISHMENTS AND OPPORTUNITIES: A REPORT FROM THE NATIONAL PERSPECTIVE

Richard A. Millstein

I am very pleased to be here with you again this year to share in the exhilaration that characterizes this important meeting and to share the exciting and fruitful efforts in which we have been engaged.

At this time last year, I spoke to you about the imminent reorganization which would bring NIDA, as well as the National Institute of Mental Health, and the National Institute on Alcohol Abuse and Alcoholism, within the organizational fold of the National Institutes of Health (NIH).

The mechanics of this reorganization are now well behind us; and while the challenges of the process of integration into the NIH system have not been easy, this move has conferred an important gain on our Institute and on our field: the colocation of science on the disease of drug addiction with the rest of this nation's premier biomedical and behavioral research enterprise at the NIH. By placement of NIDA alongside the other NIH Institutes, Congress affirmed the status of drug abuse as a disease which should command the same degree of compassion and scientific dedication--and funding--as any other disease. NIDA has moved forward and is, I believe, stronger and more resilient than ever, and better equipped to accomplish our central mission of supporting scientific research of the highest excellence. I feel a genuine and growing sense of optimism with regard to NIDA's future and for the future of drug abuse research.

In keeping with this promising opportunity, at last year's meeting I spoke to you on the State-of-the-Institute in relation to a "Focus on the Future: A Steadfast Commitment to Research." Today, with the experience of 12 months of nearly continuous change, I come before you not only to reaffirm that commitment but also to convey to you what I see as critical to our ability to ensure this commitment. NIDA and the research community we represent can and will capitalize on the unprecedented and unparalleled scientific opportunities presented by dint of your work, and the excellence of the science will be matched with the funding it should command to optimally fulfill the promise, only if we--all of us--find ways to enlighten the American public--and here I include prevention and treatment practitioners, foundations, the media, and policy-makers--on the value and the far-reaching benefits of research on drug abuse--now, in this coming year, not "by the year 2000." For without the necessary resources, these opportunities to significantly further our mission to improve the public health, no matter how vital, will remain unrealized. NIDA-funded research has consistently shown that, like many other diseases, drug addiction is preventable, measurable, and treatable. And, as with research into other

diseases, long-term, stable funding in the addiction sciences can--as it has in the past--yield improvements in the quality of life for millions of affected individuals; decrease health care costs; and help the Nation fight serious communicable diseases such as AIDS and tuberculosis. But we have to tell that story.

And unlike other health Institutes whose areas of research receive substantial research support from the private sector, NIDA is, in essence, nearly the sole provider of our Nation's drug abuse research--supporting 88% of that conducted in our country. This means that when the Congressional Budget Office recommended, just 3 or 4 months ago, that scientists funded by NIH can find other means of support--for a five-year so-called "savings" to the Nation of 1.7 billion dollars--we must conclude that they have not been well enough informed about the nigh absence of alternative funding sources for drug abuse researchers and of the value of the work in which we engage. It also means that when budget appropriations for NIDA reach a stalemate, so does drug abuse research. And this against a backdrop of substantial declines in the past few years already. Just from 1990 to 1993, as but one measure, the number of competing RO1 grants supported by NIDA has suffered a 15% decrease, from 267 in 1990 to an estimated 224 this year. We have to tell that story as well.

Now some promising news.

In the past few months many national figures have been suggesting that the President and the Office of National Drug Control Policy review the allotment of Federal drug abuse control expenditures. Today, over 60% of the Federal budget authority for drug abuse is directed to supply reduction; less than 33% to prevention and treatment activities; and only about 4% to drug abuse research--this, despite the fact that research provides essential information about the causes of drug addiction while assessing new prevention strategies and treatments for its consequences. Reallocation of just 1% of the 13 billion dollar Federal drug abuse expenditure--130 million dollars--into the research budget of NlDA would allow for the development of advances that are critical to the provision of long range solutions to the multitude of drug abuse problems this Nation daily confronts--and would not increase the nation's budget deficit.

NIDA, under legislative authority, has developed a bypass budget request of more than $500 million, which would allow the enhancement of research that promises to have a major impact on the health problems of today by increasing the 4% now dedicated to research to 5% through reallocation of existing funding. This is the recommendation made by CPDD in its testimony before the House Appropriations Subcommittee last February, and by other groups and individuals as well.

Further, it is not the research community alone that is promoting additional funding without affecting the deficit. Just last month, Senators Tom Harkin and Mark Hatfield, Chairman and ranking minority member, respectively, of the Senate's HHS Appropriations Subcommittee, proposed a medical research trust fund based on a 5 dollar per month set-aside from every family's health insurance premium. Based on an expectation of 100 million policies, this amounts to $6 billion each year. Under

their proposal these funds would be allocated to NIH institutes based on each institute's percentage of the total NIH appropriation.

In addition, beyond the one percent reallocation proposal and the medical research trust fund proposal, we are aware of an increase in consensus and petition letters written to Congress in support of increased NIH funding.

So the proposals are out there. But--how will they fare?

To be optimally persuasive in terms of fiscal impact, the enormous contributions that research conducted and supported by NIDA has made in many areas must be conveyed in a meaningful and effective manner to the American public. Admittedly, this is not an easy task. We often lose sight of the fact that the scientific terminology and the technically complex ideas that are commonplace in our working environments often have no measurable impact on those for whom they are essentially unfamiliar. There is an art to the type of translation these concepts require which we must all strive to master. Enlightening the public about the very positive outcomes of investing in drug abuse research, particularly about its "real world" applicability to prevention and treatment programs, is truly an investment in the future of drug abuse research. It is up to those of us who are most knowledgeable to create a clearer understanding of how long-term activities in a laboratory can improve the quality of life on a personal and individual basis--for a child, a brother or sister trying repeatedly to get off cocaine, or for the coworker whose excessive absenteeism places an undue workload on other staff and financial burden on the company. The sense of excitement that each of us at this meeting has about the findings and the promise of research in drug abuse is what we must effectively impart to others.

Although perhaps one of the most difficult areas to communicate effectively, NIDA's basic research program has generated some recent developments that give us cause to raise the public's level of enthusiasm. These findings are important because they represent outstanding science that is literally pushing the frontiers of basic research, especially in the cellular and molecular neurosciences. And they will likely bear great fruit in revealing the biological bases of drug addiction--and also in the broader biomedical sciences--and thus have potential for further enhancing treatment of drug abuse.

For example, NIDA grantee Dr. Christopher Evans and his colleagues at UCLA reported on the cloning of the delta opioid receptor gene in the December 18, 1992 issue of Science. The same issue reported also on the work of Dr. Brigitte Kieffer at l'Ecole Superieure de Biotechnologie in Strasbourg, France on the molecular characterization of a delta opioid receptor by expression cloning. As you know, investigators discovered endogenous opioid peptides roughly 15 years ago. In the laboratory, however, these compounds have remained ligands in search of receptors--receptors which at times seemed grail-like as researchers pursued the quest for their genes. Now, the cloning of the first opioid receptor gene has opened the way for discovering others, and for conducting more rigorous studies on their role in fundamental cellular processes, such as neuronal control and, perhaps, even

development. This work, in turn, offers promise for enhancing our understanding of the biological mechanisms associated with addiction and withdrawal, pain and analgesia, and other processes of the nervous system--ultimately, for developing better medications to treat addiction, pain, and other disorders. Drs. Evans and Kieffer will be reviewing their research at a symposium today.

As another example, Drs. William Devane and Raphael Mechoulam reported that in their NIDA-supported work at the Hebrew University of Jerusalem they found a compound which they call "anandamide" (a Sanskrit word meaning "eternal bliss") that behaves like an endogenous ligand to the cannabinoid receptor. This compound is derived from arachidonic acid--a fatty acid component of cell membranes. This research is almost a mirror image of the opiate research, which has focused on the search for the receptors of known endogenous ligands; the cannabinoid studies have centered on the pursuit of endogenous ligands for a receptor cloned just 2 years ago.

This work is significant for several reasons. It offers promise of studies on an entirely new neurochemical system and, ultimately, perhaps even insights into the processes associated with learning, memory, mood, and perception. Medically, it also opens new avenues in the search for analgesics, antihypertensives, anti-nausea drugs, and medications for treating glaucoma. This work will be discussed in a symposium tomorrow.

And yet another development relates to nitric oxide--featured as "Molecule of the Year" for 1992 by Science magazine. Nitric oxide received this honor because of the burgeoning numbers of studies in 1992 implicating the simple--or is it not so simple?--gas in a host of biological processes ranging from digestion to blood pressure regulation. Among the most intriguing of its roles to emerge has been its possible function as a novel neurotransmitter. NIDA is pleased to have supported--through the laboratory of Dr. Solomon Snyder at Johns Hopkins University--much of the original work which has spurred studies of nitric oxide's role in the nervous system.

Dr. Snyder and his colleagues isolated and characterized nitric oxide synthase, the enzyme responsible for making nitric oxide in the body, and studied its distribution in the brain. From their work came postulates of nitric oxide's role as a novel neurotransmitter which performs its actions through diffusion rather than packaging and release in traditional synaptic vesicles. Dr. Snyder has postulated that nitric oxide may be but one member of a family of novel neurotransmitters; he and his colleagues have presented evidence suggesting that carbon monoxide may also function as a neurotransmitter.

NIDA is very excited to be playing a significant role in discoveries concerning nitric oxide, because a picture is emerging which reveals that this molecule has a much larger role in basic biological mechanisms than any of us might have imagined even a very short time ago. For example, in their studies on neurotoxicity, Dr. Snyder and his colleagues already have implicated nitric oxide as a possible mediator of some of the toxic phenomena associated with the HIV-1 virus.

In other NIDA-funded work, Dr. Gavril Pasternak and his colleagues at the Memorial Sloan-Kettering Cancer Center and Cornell University Medical College have some very intriguing findings suggesting that morphine tolerance in mice can be blocked by an inhibitor of nitric oxide synthase.

Of course, since I spoke with you last year, there have been, in addition to these developments from NIDA's basic research program, many, many significant findings in drug abuse research across the spectrum of basic and clinical science. I will not, as I did last year, present a comprehensive overview. But as just a sampling:

- While at the Montefiore Methadone Maintenance Treatment Program in the Bronx, NIDA grantee Dr. Peter Selwyn demonstrated that prophylactic treatment for active tuberculosis is effective with drug abusers attending a methadone treatment program. Injecting drug users who are coinfected with HIV and the tubercle bacillus are at high risk for developing active TB. Dr. Selwyn found a significantly decreased rate of development of active TB among those patients who received and complied with the recommended 12-month TB treatment regimen.

- NIDA grantee Dr. Thomas McLellan at the University of Pennsylvania has shown that the treatment success rate of methadone maintained patients is greatly enhanced with the addition of medical services, social work assistance, family therapy, and employment counseling and job training. Improvement has been seen in terms of decreased cocaine and alcohol use, psychological problems, and crime rates, and increased employment.

- And at Emory University, Dr. Claire Coles' recently-completed study of fetal growth and neonatal behavior relative to prenatal exposure to cocaine and alcohol reported no evidence of dysmorphia or physiological withdrawal in a sample of full-term neonates. This study suggests that many of the problems seen in cocaine-exposed infants may be due to cocaine's ability to cause premature birth, or to the effects of other drugs used in addition to cocaine.

PRIORITY PLANNING

So we have the scientific opportunities. We may have the opportunity to compete for additional funding to support them. We are recommitted to do even more to educate and enlighten others and to infect them with our passion and enthusiasm. And now the mode of transmission.

As I described to you last year, NIDA staff have been engaged in the development of S-year research plans in 7 areas: neuroscience, medications development, nonpharmacologic treatment, AIDS, maternal and fetal effects of drugs of abuse, epidemiology and prevention, and research training and career development. Through the development of these NIDA 5-year plans so many of you have been so instrumental in shaping we now will be able to convey not only to drug abuse researchers, but also to practitioners, to Congress, and to the lay public our current and

future research priorities, founded on scientific promise and public health need, and our vision for the future. These plans provide convincing documentation that drug abuse not only is a serious and costly health problem in its own right, but one that impacts heavily as well on such medical and social problems as HIV infection and AIDS, hepatitis, tuberculosis, violence, the productivity of our workforce, and the educational abilities of our children--whether born to an addicted mother, raised in an environment permeated by parental drug abuse, or taking drugs while attending school. They are reflective of our belief that, if these health and social problems and their high associated economic and human costs are to be reduced, we must address the prevalence of drug abuse and its consequences--through research. They respond to our legislative mandate, the scientific opportunities available, and the urgent need for drug abuse research.

INITIATIVES FOR FY 1994

Now that I have presented this optimistic scenario, let me temper it--but just a bit. If there was a pre-eminent theme to my talk at Keystone last year it was "let a hundred flowers--research flowers--bloom". Today I am saying that if, despite fiscal stringencies, we are to do well in the competition for dollars, we must emphasize but 2 or 3 major initiatives, and emphasize these consistently and repeatedly. This does not gainsay the need for growth in other areas--but is strategically necessary. So this morning I would like to share with you a few particularly promising initiatives in drug abuse research which we have under way or are about to launch in the coming year. Through these initiatives NIDA hopes to capitalize on recent technological advances and treatment research methodology to the benefit of urgent public health need. They include:

- a new initiative to translate the basic neuroscience accomplishments and bring them into the clinic;

- a continuing priority on medications development for drug addiction and dependence, with a focus on medications for cocaine abuse and on medications that do not cross the placenta; and

- a second new initiative, to validate behavioral and psychotherapies for drug abuse and drug addiction.

Clinical Neuroscience of Drug Addiction

The first new initiative relates to human neuroscience. NIDA-funded research using animal models has clearly demonstrated that drug addiction directly involves neuronal circuits which mediate the pharmacological and psychological consequences of drug abuse. This evidence comes at a time when emerging brain imaging technologies permit the visualization of the structure and activity of the brain during the administration of drugs of abuse, as well as the interaction of drugs, drug abuse medications, and behavioral therapies in the living brain.

These developments point to a compelling opportunity for NIDA to expand its neuroscience program in the area of human studies. Expanded research will lead to answers to the most central and baffling problems of addiction, such as why drug-induced euphoria occurs; how environmental variables and cues, learning, and memory interact in the addiction process; what biological and behavioral mechanisms are responsible for craving, abstinence, tolerance, and dependence; and why it is so difficult to break the addiction cycle.

In fiscal year 1994, NIDA plans to expand its neuroscience program in the area of human studies with the Clinical Neuroscience Initiative. The Initiative will develop, integrate, and validate non-invasive technologies for use in drug abuse diagnosis and treatment. As part of this approach, investigators will measure neuroanatomical, chemical, and physiological parameters associated with drug use during the various stages of addictive disorders--including craving, tolerance and dependence, withdrawal, abstinence, and relapse.

For various drugs and use patterns, studies will focus on identifying neuronal systems associated with reinforcement and brain reward, the biochemical mechanisms through which they function, and the effects of drugs upon these systems. In addition, this Initiative will assess the full range of brain development and maturation, including possible relationships between early or chronic drug use and brain function in later life and the onset of subsequent neurological disorders. Particular emphasis will be placed on studies assessing the prenatal and perinatal effects of in utero drug exposure through a variety of cognitive function and behavioral tests. NIDA is proposing to support regional multidisciplinary neuroscience research facilities which would have as their focal point state-of-the-art neuroimaging facilities. The research at these centers would include both animal and human studies, with the emphases on prevention and treatment. In addition, the imaging facility would be used in this clinical setting to evaluate the efficacy of various treatment modalities, including medications and behavioral approaches. The clinical neuroscience centers would be developed in sites that would allow us to attract trained scientists and clinicians from other related disciplines, such as psychiatry, neurology, cognitive neurobiology, and computational neuroscience--experts who might not otherwise consider drug abuse research as a career. Perhaps most importantly, we propose that each site have contained within it a state-of-the-art drug treatment facility, to provide care for patients in addition to conducting research.

Medications Development for Drug Addiction and Dependence

As for medications development, a continuing priority, as you know, of the thousands of medications approved for marketing in this country, only two have been approved for drug abuse indications: methadone, available for a quarter of a century, and naltrexone, approved for marketing in 1984. A third medication, LAAM (levo alpha acetyl methadol) was reviewed just last week by the FDA's Drug Abuse Advisory Committee, and, I am delighted to report, received unanimous approval. This recommendation now goes to an FDA Review Group for action.

Rapid approval is anticipated for LAAM's formal New Drug Application (NDA) to be filed next week, and after the 3 to 4 months for required regulatory changes to occur and for the pharmaceutical manufacturer to launch the product for clinical treatment of opiate dependence, a third medication will be available--our programs's first tangible success in terms of a newly marketed pharmaceutical for a drug abuse indication.

LAAM is not intended to replace methadone; rather, its use is intended to provide the treatment community with more options and improved treatment matching for patients. We hope that this will help us progress from serving 100,000 individuals in need of treatment for opiate dependence to 200,000.

We expect to follow the availability of this third medication with buprenorphine and clonidine within the next 18 to 24 months. NIDA is now conducting a 12-site, 720-subject double-blind clinical trial to study the effectiveness of buprenorphine at various doses (1, 4, 8, and 16 milligrams) and plans to develop buprenorphine as well as a buprenorphine-plus-antagonist medication to lessen the risk of diversion.

LAAM, buprenorphine, and clonidine all, of course, are treatments for opiate dependence. With the recent isolation and cloning of the cocaine receptor, along with the availability of new brain imaging technologies, it is now promising to develop antagonist medications for cocaine. Such medications are critical because of the truly completely overpowering nature of drug craving and the effects of drugs of abuse on the brain and alteration of behavior. Medications that reduce this craving will also allow nonpharmacological treatments to strengthen or reinforce the decision-making processes of the brain.

Teams of structural and molecular biologists are now prepared to modify the amino acid sequence of the receptor protein to alter the binding of cocaine. The newly synthesized candidate medications will then be administered and brain imaging technologies used to visualize the effects of the medications on brain structure and function. Our increased understanding of the interrelationship between the brain and behavior will allow us to modulate and influence various systems and receptor proteins that can prevent the profound effects of drugs of abuse.

Building on the successes of the medications development program and fundamental research on the mechanisms of action of drugs of abuse, another priority that can now be explored is the development of medications that do not cross the placenta or are not active in the fetus, so that drug dependent and addicted pregnant women can receive drug treatment without major influence on the developing fetus.

Behavioral Therapies

Our ultimate goal is to successfully merge the best pharmacotherapies and the best behavioral therapies into comprehensive, integrated treatment programs for various types of addiction. That brings me to our second new initiative in 1994, which relates to the behavioral and psychotherapies.

Drug addiction is a complex disease with strong, indeed critical, behavioral and psychosocial determinants, and such factors must be addressed in the treatment of the drug abusing patient. Behavioral therapies, including a range of psychosocial interventions, are the most frequently administered treatments for drug addiction, and they remain the only available treatment approaches for many drugs of abuse. Behavioral interventions also serve as adjuncts to pharmacological treatments, addressing concomitant interpersonal and environmental problems and facilitating medications compliance. But while considerable progress has been made over the past decade in developing promising behavioral therapies, treatment retention and relapse too often remain problematic.

Systematic research on psychotherapy and behavior therapy for drug addicts is in its early stages. Although many forms of therapy exist, most were not developed or tested specifically for drug abusers. There exists no counterpart for the behavioral and psychotherapies of the FDA role in approving pharmacotherapies for the treatment of drug abuse disorders. NIDA therefore plans to support a Behavioral Therapies Initiative to apply the same rigorous testing processes to behavioral therapies.

This Initiative will build on the knowledge gained from basic behavioral studies to identify, formulate, and systematically test promising existing psychotherapeutic, behavioral, and counseling interventions, as well as to develop and test new therapeutic modalities. Particular effort will be made to develop protocols for matching specific types of patients to particular therapies and to develop new counseling strategies for treating comorbid drug abusers. The Behavioral Therapies Initiative includes four phases:

- Phase I involves identifying promising research findings relevant to drug abuse treatment, generating and formulating new psychobehavioral therapies, operationally defining the therapies in manuals, and pilot testing and modifying the therapies.

- Phase II continues NIDA's program of small-scale efficacy testing of promising therapies and undertakes the replication, at other sites, of studies with positive results.

- Phase III consists of large-scale testing of therapies that have been shown to be efficacious in more than one controlled clinical trial. This involves rigorously controlled, centrally coordinated, multi-site clinical trials of promising therapies in community-based treatment programs,

- In Phase IV, results of the efficacy studies will be evaluated by panels of experts, and where substantial evidence for efficacy is demonstrated, the therapy will be packaged in a way that practitioners can utilize it most effectively. Also, through our research dissemination and technology transfer programs, NIDA will develop videotapes, curricula, and other means for translating the results of the research into clinical practice.

DEVELOPMENT OF DRUG ABUSE RESEARCHERS

There is so much I cannot in any one presentation talk about. I have only touched on our AIDS program and our expanded research on drug users and tuberculosis. I have barely noted the increasing sophistication of our prevention research in achieving and sustaining behavioral change. And there is so much more. Nonetheless, and despite time strictures, I must address NIDA's long-range planning to ensure an adequate supply of "intellectual capital"--well-trained biomedical and behavioral researchers critical to the long-term success of addiction research--the sine qua non for a strong research program to flourish. NIDA's current training and career efforts are aimed toward increasing the pool of students interested in drug abuse research; providing adequate resources to support the training of those individuals who choose to pursue careers in this field; increasing access to research support and training for special populations and research scientists; and improving the retention of trained investigators, especially clinical investigators.

In 1992, we supported 275 full-time training fellowships for pre- and postdoctoral study; 73 investigators were awarded individual fellowships and 202 were supported through institutional training grants. But the cadre of drug abuse researchers is aging; of persons submitting competing research grant applications, those between age 46-50 are increasing while those age 3.5 and below are on the decline. If we do not intervene now, this trend will severely impede progress and advances towards our understanding of addiction, and thus towards better drug abuse prevention and treatment.

We have just in the past few weeks been saddened by the loss of two virtual giants in the field of drug abuse.

- In May, Dr. William R. Martin died at his home in Kentucky. Dr. Martin spent more than 40 years in neuropsychopharmacology, and served as Director of NIDA's Addiction Research Center from 1963-1976. His work focused on the addictive nature of narcotic analgesics, and led to the development of new analgesics which have greatly increased the safety of treatment for chronic pain. His prescience was sometimes uncanny, evidenced by the redundancy model of drug tolerance, multiple opiate receptors, and the critical interaction between drug dependence, psychopathology, and criminal behavior. At the time of his death, he was an anesthesiology professor at the University of Kentucky College of Medicine, where he also conducted research on drug addiction.

- And on June 2, Dr. Daniel X. Freedman passed away in Los Angeles. A pioneer in psychopharmacology, in the 1950s Dr. Freedman demonstrated the link between hallucinogens and serotonin. He was the first to identify elevated serotonin levels in the blood of autistic patients, thus establishing a biological basis for the condition; and was among the first researchers to describe how stress affects the brain and how the brain plays a role in allergy symptoms. Dr. Freedman had been a past president of the American Psychiatric

Association and, since 1970, had been editor of the American Medical Association's Archives of General Psychiatry.

These outstanding individuals will be sorely missed by those of us who knew and worked with them and comprehend the extraordinary contributions they made during their lifetimes and that will continue to have lasting benefit in the years to come. They led by the examples of their industry, insight, and perspective, and by their role as mentor to so many drug abuse researchers. Their deaths make us keenly aware of the necessity to foster the kind of training that produced such superlative researchers, and to which they were so committed.

In fiscal year 1994, NIDA plans to initiate a comprehensive Clinical Research Training Program which will expose students at the undergraduate level to research, involve students at the graduate and post-graduate level in clinical drug abuse research, train clinicians in drug abuse research methods, and attract preventive medicine residents into clinical research involving substance abusers. This will help train individuals who can directly translate NIDA's research advances in neuroscience, pharmacotherapy, and behavioral sciences into more effective prevention and treatment strategies.

As with clinical research, epidemiology is a shortage area. Training opportunities in descriptive and analytical epidemiology and methodology development and improvement will be expanded for fellows pursuing research in the epidemiology of illicit drugs and nonmedical use of licit drugs.

Further, to fill the demand for expertise in the complex area of medications development, NIDA plans to establish a Career Development Initiative that involves trainees with both private industry and the FDA. This linkage will provide individuals with highly specific, intensive, and comprehensive research and regulatory experience that will maximize their effectiveness and productivity following the training period.

WOMEN AND MINORITIES IN DRUG ABUSE RESEARCH

In addition, NIDA will continue its support of mechanisms to promote the recruitment and retention of women and minorities to drug abuse research. For example, NIDA's Special Populations Program, designed to help minorities participate in NIDA's research activities, includes implementing training to increase the number of ethnic minority drug abuse researchers, and promoting support of more ethnic minority drug abuse research.

We also need to learn more about the subpopulations that comprise our research community and to recruit more researchers in this area. Last summer, NIDA convened a meeting of ethnic minority researchers to recommend a research and training agenda for the Institute. The Special Populations Program has also sponsored meetings with groups such as National Asian Pacific American Families Against Substance Abuse, and with representatives including presidents of Historically Black Colleges and Universities, the National Medical Association, colleges and schools of clinical pharmacy, and meetings around African American Issues involving Morehouse

School of Medicine, Drew Medical School. Hopefully, this will lead to enhanced participation in NIDA's research and training programs, increased membership on IRGs, and greater employment of minorities within NIDA.

TECHNOLOGY TRANSFER

Finally, we come to technology transfer. Continuing and improving the research process of discovery and the subsequent application of findings to practice provides our country's best opportunity for continuing to reduce the demand for illicit drugs and to reduce the burden of HIV infection and AIDS. NIDA's Technology Transfer Program provides such a mechanism for enabling researchers to transmit their study findings to prevention and treatment practitioners, and for practitioners and program directors to transmit to researchers their clinical knowledge, wisdom, judgments, and needs. These insights will serve to define and improve new research proposals as they are developed and will help to keep our research relevant to the practitioner community.

I hope many of you already are aware of NIDA's **Second National Conference on Research and Practice - An Alliance for the 2lst Century,** to be held at the Washington, D.C. Renaissance Hotel, July 14-17. This is our second drug abuse technology transfer conference designed to familiarize drug abuse practitioners from communities nationwide with promising, research-based approaches that are needed to address local drug problems. The conference is expected to draw 1,500 drug abuse prevention and treatment specialists. It will showcase outcomes of NIDA-supported research on the treatment and prevention of drug abuse and drug abuse-related HIV infection and AIDS, with a special emphasis on cost-beneficial techniques and procedures ready for application in the drug abuse field. Nationally recognized experts in research--many of whom are at this meeting--as well as frontline treatment and prevention practitioners, will share their knowledge and experience in plenary sessions, research awareness seminars, issues forums, and intensive skills-building workshops. I hope many of you will be able to attend and interrelate with other researchers and prevention and treatment practitioners in this forum to capitalize on the many new and exciting opportunities for innovative and vital research in drug abuse and to expedite the transfer and application of research findings to clinical practice.

In closing, I would like to reiterate what a pleasure it is for me to be with you at this annual meeting. I welcome the chance to talk with many of you individually about your work, our programs, and our shared commitment. We at NIDA are enormously grateful to the CPDD and its members for the ardent support you have given us through the years, and, in particular, for your continued support and assistance during this time of promise but uncertainty. Your suggestions on how to improve the visibility of drug abuse research to Congress and the American public are most welcome, and I invite you to work together with us at NIDA in mastering this essential skill. I look forward to the challenges we will face together in the coming year and to the many exciting prospects and opportunities that lie ahead.

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LINKING RESEARCH AND SERVICE DELIVERY: THE UNIQUE MISSION OF THE SUBSTANCE ABUSE AND MENTAL HEALTH SERVICES ADMINISTRATION

Mary A. Jansen

I want to thank you for inviting the Substance Abuse and Mental Health Services Administration to participate in this year's College on Problems of Drug Dependence. I bring greetings from Dr. Elaine Johnson who is our Acting Administrator and who has participated in these meetings in the past. She very much wanted to be here with you today but unfortunately she was called away on other business at the last moment. She asked me to convey her best wishes to you.

As you may know, the Substance Abuse and Mental Health Services Administration (SAMHSA) was established by Congress in October 1992 to provide Federal leadership for the prevention and treatment of addictive and mental disorders. It is the newest member of the Public Health Service and the primary Federal agency responsible for enhancing access to high quality alcohol, drug abuse, and mental health services for those who need them. Congress established SAMHA to: (and I quote from the Conference Report) "... fully develop the Federal government's ability to target effectively substance abuse and mental health services to the people most in need, and to translate research in these areas more effectively and more rapidly into the general health care system" (Committee of Conference, 1992, p. 127).

This legislation is historic in that, for the first time, Congress has recognized the relationship between the Nation's health and the provision of services to those with alcohol, drug abuse, and mental disorders. Indeed, there are few, if any disorders that impact upon America's health and societal well-being as do these.

Consider these sobering statistics which most of you already know all too well:

- 10 percent of adult Americans are alcoholics. Thirty percent of high school seniors abuse alcohol heavily.
- 40 percent of deaths in highway accidents are alcohol related.
- 33 percent of all HIV/AIDS cases are associated with injection of drugs, including 90 percent of HIV-infected infants and 71 percent of HIV-infected women.
- Cocaine mentions in emergency rooms increased by 25 percent from 1990 to 1991. Heroin mentions also have been on the rise.
- 14 percent of high school seniors smoke marijuana regularly.
- An estimated 40 percent of hospital admissions are associated with alcohol, tobacco, and/or illicit drugs.
- Over half of all incarcerated adults have alcohol and other drug problems.
- Nearly 25 percent of Americans have their health destroyed by cigarette smoking.

- The most recently published estimates of the cost of drug abuse to our society are from 1988 and these indicate that when we include costs for treatment, accidents, illness, death and the related costs for law enforcement and victimization, the figure stands at $58.3 billion. We have extrapolated from these data and used the Medical Price Index as a corollary to estimate the societal costs of drug abuse in 1992. The figure we have come up with is $194.6 billion. Also, consider that these figures do not include any costs related to "crack" cocaine.

SAMHSA's mandate is to provide Federal leadership for the prevention and treatment of addictive and mental disorders. Stated simply, SAMHSA's mission is to:

- Reduce the incidence and prevalence of addictive and mental disorders.

Thus, SAMHSA's unique national leadership responsibility is to:

- Test prevention and treatment approaches and policies to determine which are effective and which are not;
- Disseminate timely, accurate, objective information about effective prevention and treatment practices; and
- Provide targeted expenditures to initiate innovative or specialized ADM prevention and treatment programs that address populations in need of them.

To accomplish this mission, SAMHSA has developed a strategic framework for setting important priorities to ensure that prevention and treatment services are effective. The framework focuses on realizing SAMHSA's overarching mission by targeting four key principles: access, quality, empowerment, and data systems.

- Access refers to increasing the ability of individuals in need of ADM prevention and treatment services to obtain those services.
- Quality refers to improving the degree to which services provided to those in need are properly matched to their needs, are technically correct, and achieve a beneficial impact.
- Empowerment refers to expanding the ability of individuals, families and communities to actively participate in addressing factors that cause or sustain ADM disorders.
- Data Systems involves monitoring progress with objective data and improving effectiveness through services evaluations in order to answer the perennial question: "What works?'

SAMHSA consists of three Centers that administer the prevention and treatment services programs of the agency: the Center for Substance Abuse Prevention (CSAP), the Center for Substance Abuse Treatment (CSAT), and the Center for Mental Health Services (CMHS).

The Programs of each Center are summarized on this slide. As you can see, we are attempting to reduce the problems of drug dependence through a variety of demonstration programs and through the Block Grant to each state.

A word about SAMHSA's work in relation to health care reform seems in order at this point. Although we are all anxiously awaiting the Administration's proposal, it seems almost certain that substance abuse prevention and treatment services will be included in some way. If our field is to be credible and taken seriously, we must be able to identify the most effective and cost beneficial services. In order to do this, we have undertaken an array of scientific activities designed to provide answers to some of the most pressing questions.

I would like to outline for you the various kinds of scientific endeavors which arc underway within SAMHSA as we attempt to fulfill our Congressional mandate and answer these questions to identify the most effective practices in prevention and treatment service delivery.

First, each of our individual grant projects is required to conduct both a process and an outcome evaluation to determine the effects of the intervention delivered.

Second, each of the overall grant programs is conducting an evaluation of the projects funded within the program in order to determine the worth and impact of the program as a whole.

Third, each of our Centers is conducting a national evaluation designed to cull out the consistent findings from prevention and treatment service programs. Once completed, these findings will be synthesized into a coherent picture of what works, for whom, and under what circumstances.

These national evaluation efforts are exciting for us because these will be the first systematic attempts to provide a comprehensive picture of what is working. And AOD abuse prevention and treatment am emerging as robust fields that are beginning to generate measurable success.

Fourth. CSAP is planning to fund a National Center for Advancement of Prevention. A primary goal of the Center will be to develop standardized methodologies and instrumentation for the conduct of prevention intervention evaluations. The Center will also be charged with conducting secondary analyses, and where possible, meta analyses, on results obtained from primary evaluation efforts.

Fifth, CSAT's National Treatment Improvement Evaluation Survey NTIES) is a multi-year, multi-site evaluation of patient and program outcomes designed to assess the effectiveness of enhancements to standard treatment programs funded by CSAT's discretionary grants. This look at additive effects of such things as child care for women who need it to remain in treatment, and transportation for those who could not otherwise reach treatment sites, will afford us the opportunity to assess the costs and benefits of providing such ancillary services. In this analysis, the staffing patterns are also being looked at as a variable which may impact on treatment outcome. This study will conduct long-term follow-up on each cohort to assess the extent to which treatment effects are sustained in out years.

Sixth, both CSAP and CSAT are developing standards upon which to guide the field. Some of the areas targeted by this effort are depicted on this slide for prevention. These include developing criteria for culturally sensitive programs, developing needs assessment criteria, and identifying the elements of comprehensive prevention programs.

The topics under development within the treatment effort are outlined on this slide. These include improving treatment of drug exposed infants, screening for infectious diseases among substance abusers, and the development of simple screening instruments for AOD outreach.

I said earlier that a primary responsibility of SAMHSA programs is to generate knowledge about effective services and practices. As you may know, an Office of Applied Studies was created when SAMHSA came into being. This office is a part of the immediate Office of the Adminstrator and is responsible for carrying out the major surveys we conduct, identifying needed areas for services research, and overseeing the program evaluations carried out by the Agency.

In addition to the major national surveys conducted by OAS such as the National Household Survey, we arc hoping to continue the good work begun by NlDA on the Drug Services

Research Survey. This effort, which will be called the Services Research Outcomes Study (SROS) in Phase III, has given us good data on access, quality, capacity, financing and reimbursement, and has produced preliminary analyses of cost effectiveness. Some of the benefits of the SROS are that the Phase III data will be nationally representative, at the individual level, modality specific, longitudinal in nature, and linkable across programs, treatments, clients and outcomes. We are very excited about this effort and are hoping to be able to fund Phase III beginning in FY 94.

Another aspect of our Congressional mandate is to work jointly with NIDA and NIAAA around their services research program. We are in the process of developing an Interagency Agreement with the Institutes which will facilitate the identification of needed studies and hasten the feedback of results which can be disseminated to the field. Some beginning questions we hope to have answers to in the near future include:

- Which prevention models are most useful in building resiliency in populations at highest risk? And what are the critical components of these models in terms of their contribution to the overall effect?

- Which treatment models work best and under what conditions do the effects vary? In what combination do treatments need to be delivered to have maximal impact?

- Which financing and reimbursement systems encourage appropriate use of effective service interventions where a cost benefit ceiling becomes apparent?

- What changes or additions need to be made to existing diagnostic and procedure nomenclature and coding systems to facilitate enhanced information collection, research and service delivery?

But obtaining the answers to critical questions such as these is only the first step in fulfilling our responsibilities to the field. The next step is to transfer new knowledge to those who are developing interventions. This knowledge transfer function is one which SAMHSA takes very seriously, and we need your help if we are to be successful in our efforts to disseminate timely new knowledge. We must work together with the Institutes and all of you to achieve our joint goal of reducing our nation's dependence on drugs.

Last year at this meeting, Dr. Elaine Johnson issued a challenge to you to help us as we build this new agency into one which will truly serve all those affected by substance abuse disorders. I want to renew that challenge and tell you that I believe that if we work together we can indeed reduce the tragic loss of life and productivity due to substance abuse disorders. I hope we can count on you to help us with this task.

Thank you.

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INTRODUCTION OF NATHAN B. EDDY AWARD RECIPIENT

L. B. Cottler

It is a great privilege for me to introduce Dr. Lee Robins, my mentor and friend, as this year's recipient of the Nathan B. Eddy Award.

This award, which commemorates the legacy of Nathan Eddy since his death in 1973, is meant to be an acknowledgement of excellence in research relevant to drug abuse. A wonderful description of Nathan Eddy was incorporated into the lecture by the first Eddy awardee. Dr. Seevers. He was described as "a plain man of indomitable will, untiring zeal, and undeviating faith in the goal he sought." If these are qualities the Selection Committee uses, those who know Lee can see why the Committee chose her.

In the 50's, while changing diapers and cooking meals for her family of four boys, Lee was working on her Ph.D. thesis. In describing life as a working mom to a Radcliffe-Harvard alumnae magazine reporter, she tells the story of having her mother-in-law watch her sick children so she could defend her dissertation and remembering how her mother-in-law eventually offered to pay her to stay home and take care of her grandchildren. Thank goodness Lee said, "No."

Although Lee would have you believe that her academic achievements have mostly fallen into her lap, those of us who know her well, recognize that this is not true. On the other hand, when opportunities have fallen into her lap, she has known how to "sew a silk purse from a sow's ear." Her first contribution to the field of social psvchiatric epidemiologv, and eventually to the drug abuse field, is a good example of this. Lee was a junior faculty member in the Department of Psychiatry at Washington University School of Medicine when a psvchiatrist friend asked her if she were interested in the medical records of one of the first child guidance clinics in the U. S. which were stored in an unused city hospital room and about to be burned. She heard these records crying out for her and agreed to take them. The records were used to follow up 524 children who had been treated for behavior problems. This was the first longitudinal studv of disorders among children and the best account to date of the natural history of childhood behavior problems. The classic text entitled "Deviant Children Grown Up", described the 30 year follow-up study. Achieving a 95 percent response rate, Lee found early antisocial behavior to be the strongest predictor of later adult behavior-even stronger than social class, economic status or family background. This finding has had implications for recent work in the field with studies looking at precursors of substance abuse and dependence and the relationship between ASP and high risk HIV behaviors.

In this study, and in those that would follow, Lee pioneered new approaches in research methodology, which have been revolutionary in the fields of social psychiatry. It was in this first study that she started to use lay interviewers to collect detailed histories from respondents. She found that non-clinicians were capable of eliciting information from respondents that clinicians often found difficult, such as information on sexual behaviors, child abuse, illicit drug use and criminal activities.

The child guidance study was followed by another study on the problems of African American school children, which in turn, led to her next project, which was commissioned by the White House Special Action Office for Drug Abuse Prevention. There were concerns about a large number of returning Vietnam Veterans who had been addicted to narcotics while in Vietnam. The study was funded by the Department of Defense, Departments of Justice, and Labor, the Veterans Administration and the National Institute of Mental Health. The first wave of face-to-face interviews was conducted in 1972, a year after the soldiers had returned home. Three years later, a NIDA- funded follow-up was conducted on 93

percent of the men. Among the major findings were that the rate of opiate addiction dropped from 20 percent in Vietnam to one percent one year after Vietnam, and less than 10 percent of those addicted in Vietnam became re-addicted to opiates in the three years after returning home. This study helped clarify the natural history of heroin addiction, and demonstrated quite clearly the influence of "triggers" and "environmental cues" on drug-using behavior.

In the larger field of psychiatric epidemiology, which of course includes substance use disorders, Lee's contributions has had a profound global impact. In the olden days, psychiatrists themselves went out into the community and surveyed people about their problems.

This was obviously expensive and could lead to information bias since clinicians often have. their own biases about what line of questioning should be followed and what characteristics are most important to them. Thus, not all persons are asked the same set of questions--for example, little old ladies are not asked about alcohol, or deviant behaviors and virtually no one had the nerve to ask about sexual behaviors.

However, the Department of Psychiatry at WUMS, changed all of this with its pioneering work by developing criteria for all major mental disorders--including substance use disorders. Eli Robins, Lee's husband, who was Head of the Department from 1963-1975, brought psychiatry out of the dark ages with a new approach in psychiatry with the help of Lee. Along with this diagnostic manual of criteria, which was the forerunner to the DSM manual, an-interview was developed so that clinicans could begin to ask patients about their behaviors in a consistent way. With Lee's help, a formal interview was written, and written in such a way so that even non-clinicians could ask the questions!

Because of this pioneering work, the NIMH asked Lee to consider applying for a grant to develop an instrument which would be suitable for a major epidemiologic survey in the United States. This effort eventually became the NIMH ECA project and the instrument became the Diagnostic Interview Schedule. Lee was one of five PIs on the ECA study--the largest collaborative effort to document mental disorders in North America.

Now the DIS has been translated into over 30 languages. Due to the success of this approach, the WHO asked Lee to spearhead the development of another interview based on the ICD-10 criteria--one that could be applicable for use in many cultures. This instrument is the CIDI and already has several offspring such as the clinician assessment tool, the SCAN, the substance abuse module, and screening and computer versions. With the advent of these instruments, there is an opportunity for cross-cultural and cross-national comparisons in rates of mental disorder that has been unprecedented.

Every decade, Lee has had a major study--all have made major contributions to the field of social science, psychiatry and substance abuse. I have worked with Lee for almost 13 years. Her high standards might intimidate people who don't know her very well. But she fosters independence by demanding clear thinking, clear writing and creativity. Let me give you my personal experience. The best advice Lee ever gave me was received very poorly on my part at first. It was the week before Christmas and we were meeting over my third revision to my dissertation proposal. I was very frustrated when she told me the specific aims on my proposal were still not clear enough. She told me that I would never make it in research if I couldn't write a clear proposal. Boy, was she right!

Recently, I have observed a change in Lee's activities. She has shifted her focus from conducting her own studies to mentoring other's work. She has been a consultant to many people in this audience, including Institute Directors; thus, her recent contributions are having an even broader impact in the field as she creates the state-of-the-art

Like all "big shots", Lee has held important positions and offices. She was the first woman and social scientist on the Board of Directors of CPDD. She has been honored with many

awards, including the Paul Hoch Award, the NIDA Pacesetter Award, the Sutherland Award, and the Rema Lapouse Award. In addition, Lee is an honorary fellow of the Royal Societv of Psvchiatrists and the American Societv of Psvchiatrists. She has been a prolific writer-authoring 15 books, authoring or co-authoring 237 papers. Recently, Washington University named her University Professor--which means that she is an official treasure to the entire University.

What Lee has done for us in the field of drug and psychiatric epidemiology and social science, has been to give us a way to communicate with one another more precisely--forever. Although in the future we might revise her interview questions slightly by updating them to fit the times, her method will never be outdated. She has elevated the task of writing questions, asking questions and devising methods for conducting studies to a precise science. Those of us who have collaborated with her on instrument development are impressed with her perspicaciousness. Her line tooth comb is the finest I have ever seen.

In addition to her numerous academic achievements, Lee promotes a warm family atmosphere at work, is a fantastic cook, especially of her native New Orleans cuisine, a great seamstress of her grandchildren's Halloween costumes, a fun traveling companion and a whiz at the New York Times crossword puzzles.

The fields of social science and psychiatric epidemiology are celebrated today as Lee Robins is awarded the Nathan B. Eddv Award. The women in the audience can be proud, too; she is the second female recipient of this award but the first to receive it solo. On behalf of the College on Problems of Drug Dependence, I am happy to help present the Nathan B. Eddy Award to Dr. Lee Robins.

AFFILIATION:

Washington School of Medicine
St. Louis, Missouri

THE NATHAN B. EDDY LECTURE: CHALLENGING CONVENTIONAL WISDOM ABOUT DRUG ABUSE

L. N. Robins

It is a tremendous honor to be the Eddy awardee. I have held in awe both Dr. Eddy's name and the dedication of the Committee on Problems of Drug Dependence (CPDD) to the research areas he initiated since I joined the Committee in 1972. I have also been more than awed by the people who have received this honor before me. This award has recognized those who gave us new insights into the working of the brain, cracked the secrets of the effects of chemicals on the nervous system, demonstrated that animals of many species can detect the rewards of drug use, feel the pain of withdrawal, and develop conditioned responses to drugs. These findings in turn have made it possible to predict how human beings will respond to new drugs to which no man has yet been exposed.

As I looked over the achievements listed by previous awardees, I wondered how the work of an epidemiologist could compare with these. I was not just afflicted with personal modesty. I once served on an ill-fated Medical School committee created to select an epidemiologist as head of a department of population medicine. Instead of choosing a leader, we witnessed the destruction of the department by a distinguished bench scientist who said, "Why hire an epidemiologist; he'll never get a Nobel prize." Probably none of us ever will, but for me the Eddy award is its closest relative, and I am thrilled to be its recipient.

When I asked myself what my work in the epidemiology of substance use and abuse has accomplished, I decided that probably its importance was in challenging the conventional wisdom with findings that have stood the test of replication across diverse populations studied in different ways and in different eras. I wish I could claim that these findings had then been reflected in public policy, but changing public views requires more than scientific evidence.

Still, the public view is not exactly as it was at the time I began working on substance abuse almost 30 years ago, early in the history of the drug epidemic that is still with us. At that time, everyone "knew" that marijuana was a stepping stone to hard drugs, that heroin was the most dangerous drug of all, that exposure to it rapidly and inevitably led to addiction, with characteristic craving that required engaging in violent crime to satisfy its demands, and that addiction was virtually permanent without long-term, preferably residential, treatment. This "information" came almost exclusively from studying people in treatment, because everyone also "knew" that you couldn't interview non-patients about illegal behaviors because they would lie.

I became involved in studying substance abuse as a by-product of following antisocial children into adulthood. Fist I studied the adult lives of child guidance clinic patients seen in the 1920s and compared them with a control group selected from school records and matched for neighborhood, race, and IQ. Drug abuse was one of the outcomes I looked at, but it was rare in that cohort. Based on personal interviews and the collection of many records (arrests, welfare, psychiatric care, etc.),

we found excess alcoholism, crime, poor work histories, and marital breakup among the patients referred for antisocial behavior. We also found that both ex-patients and controls were quite frank in interview, as revealed by agreement of their statements with what we found in records they did not know we had.

A STUDY OF YOUNG BLACK URBAN MEN

To validate the results, I repeated the study in a small sample of black St. Louis-born schoolboys born 20 years later. These men reached adolescence shortly after World War II, when drugs had entered the black ghettos but had not yet reached the white middle class. I studied their outcomes in their early thirties. They reached that age in the mid-1960s. by which time the country was frantic about the impact of drugs on the young. We interviewed them and again collected a great variety of records for them -- police, psychiatric, school, military. We found the men to be extremely honest. Only one man for whom we found a record of a drug-related arrest failed to tell us in interview that he had used illicit drugs.

Half of these young men reported having used marijuana, and 10% said they had been addicted to heroin. Their drug use typically began with marijuana at about age 15. Most used no other drugs, but if they did go on, they progressed first to barbiturates, then to amphetamines, and finally to heroin.

Popular stereotypes about who would use and what the outcome would be did not hold. The users were not the dregs of society. In fact, they came from the slightly more affluent families and had slightly higher IQs than the non-users. They obtained their drugs from friends, not from an evil pusher in the schoolyard. They did more often have a history of antisocial behavior before they began drug use. Also they often did poorly in school, despite their good IQs, and eventually dropped out (Robins and Murphy, 1967).

Their drug use fit the model of an infectious disease epidemic rather well. Those at risk had friends who were users. Social isolates and those so deviant that they never entered high school were protected against drug use.

The most surprising finding was that almost all the men who said they had been addicted to heroin were free of heroin use throughout the year of interview, although very few had had any treatment (Figure 1).

The other big surprise was that virtually the only men still using marijuana at age 31-33 were those using heroin (Figure 2). Although drug use had begun with marijuana, the progression was not a stepping stone pattern. Rather it was a pattern of accretion, with the addition of new drugs preserving the drug used previously.

These results were surprising, but they were based on a small sample from a single birth cohort born in one city. Were the findings true elsewhere? My chance to find out was provided by Dr. Jerome Jaffe.

Figure 1

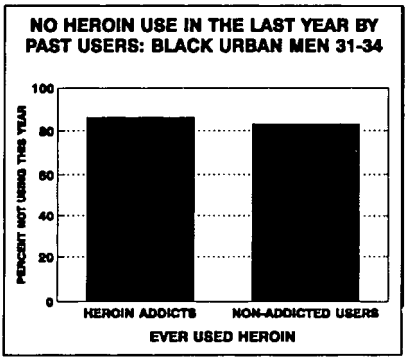
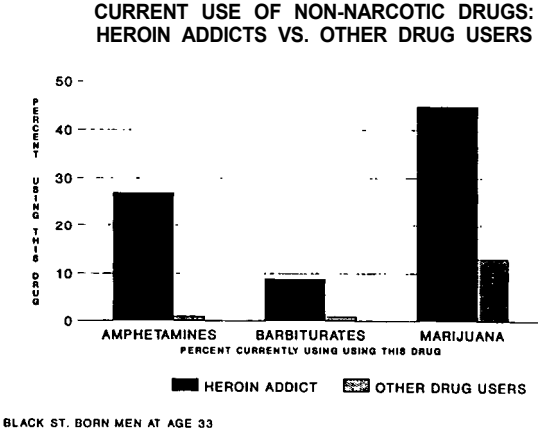


Figure 2



VIETNAM VETERANS

In June 1971, at President Nixon's request, Jerome Jaffe went to Vietnam and established a urine screening program, which required that any man due to leave Vietnam be tested and have a "clean" urine before boarding the airplane. Urine initially positive by the FRAT test was to be re-tested with gas chromatography. If the test was again positive, the man was to be sent to detoxification for about a week, and his urine re-tested before he was allowed to leave the country. As soon as Dr. Jaffe returned from Vietnam, he invited me to design a study to estimate the size of the problem both in Vietnam and after return. To do so, I selected 900 men who, in

September of 1971, when they returned from Vietnam, had been Army enlistees. We enriched the number of heroin users in the sample by choosing approximately half this sample from among those for whom the Surgeon General's office had a record showing a positive urine test at departure. Military records were then checked to verify departure dates. From Selective Service records, we drew a sample of draft-eligible civilians who did not serve but matched the veterans with respect to age, region lived in, and education at the time the veterans entered service.

We had a remarkably high interview completion rate, 96% when the veterans had been back 8-12 months. Our completion rate for a second interview at 3 years after their departure from Vietnam was 94% for the men previously interviewed, and the same rate for the control sample.

We also had access to all the record information we needed from the military and the Veterans Administration. And we collected urines at the end of each interview. These checks on what the men told us showed impressive honesty: 97% of those whose military record showed narcotics use told us about their use while in service, and tests of urine samples collected at the end of the interview showed no higher rates positive for current use than did their self-reports given before they knew they would be asked for a urine sample.

Narcotic use in Vietnam

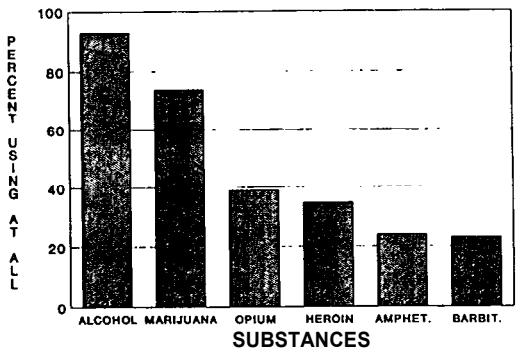
The findings were remarkable. We estimated that almost half (45%) of Army enlisted men in Vietnam in 1970-71 had tried narcotics; 34% tried heroin and 38% tried opium. While fewer used heroin or opium than alcohol or marijuana (Figure 3). narcotics were used at a truly astounding rate. These figures reflect the fact that narcotics were cheap and pure, so pure that there was no need to inject them, a method which would have repelled many soldiers. Instead heroin could be mixed with tobacco and smoked. That opium was used so widely was a particular surprise; the press had reported only on heroin use.

Physical dependence was common. Twenty percent claimed to have felt strung out or addicted to narcotics, almost half of those who used narcotics at all. While 20% of a general population being dependent was a truly amazing figure, it is still notable that half of those who used did not become dependent.

Almost 11% of these Army enlisted men's urines had tested positive at departure. This meant that half of the men who reported being dependent in Vietnam had stopped their use at least 3 or 4 days prior to their scheduled departure. We asked men detected as positive why they had not quit, and found that it was not always craving for the drug or fear of withdrawal that explained their positive urine. Some had received their departure orders less than the 3 days ahead needed for their urines to clear; others were ignorant about the chemistry involved, and knowing they were to be tested for heroin, switched to opium, and of course tested positive. Thus more than half could have stopped their narcotic use on their own, without help in detoxifying, had they had the information they needed.

Figure 3

SUBSTANCE USE IN VIETNAM



The orderly pattern, describable as a Guttman scale, in which no one uses an illicit drug without also using the legal drugs alcohol and tobacco, and no one uses a "hard" illicit drug without also using a "softer" illicit drug (Kandel and Faust, 1975), was turned topsy-turvy in Vietnam. While for these very same men both before and after Vietnam, heavy alcohol use was almost a prerequisite for narcotic use, in Vietnam, narcotics and alcohol were inversely correlated (Figure 4). This may have been a result of military rules against selling alcohol to soldiers under age 21; for the average enlisted man, who arrived in Vietnam at age 19, heroin was more available than alcohol. The pattern was also reversed for amphetamines. Before service, amphetamines were often used by men who used no opiates, but the reverse was not true. In Vietnam, amphetamines were essentially used only by users of narcotics. (Remember we did not study Marines, whose Green Beret divisions received amphetamines as part of their military issue kit.) These pattern reversals show that a drug's position in the sequence has more to do with its availability and cost than either with its intrinsic qualities such as addiction liability, or with popular beliefs about its dangerousness.

What about the impact of the childhood behavior problems that had predicted drug use so effectively in my study of young black men? One might have expected that the much greater availability of drugs in Vietnam and the stresses of serving in a war would dwarf the link with childhood deviance. But they did not. The relationship between early behavior problems and drug use held in Vietnam. We counted occurrence and severity of 5 pre-service behaviors: fighting, truanting, drunkenness, arrest, and school expulsion. Each was scored as not present, present mildly, or present at a serious level. Both a larger number and greater seriousness of these behaviors increased the risk of using narcotics in Vietnam (Figure 5).

Figure 4

IN VIETNAM, NARCOTIC USE AND DRINKING WERE INVERSELY RELATED

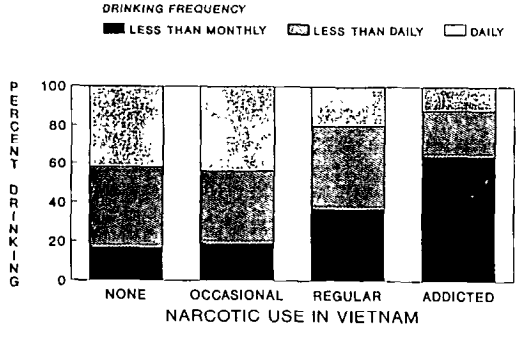
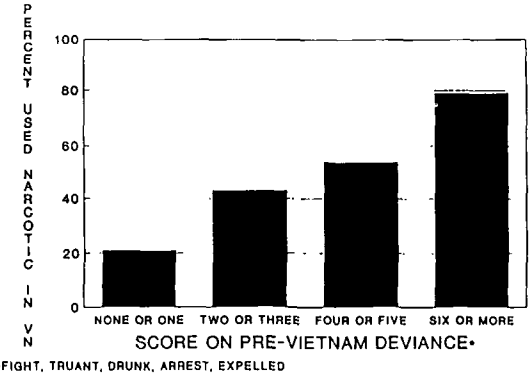


Figure 5

BEHAVIOR BEFORE VIETNAM AND NARCOTIC USE IN VIETNAM



•FIGHT, TRUANT, DRUNK, ARREST, EXPELLED

A history of pre-service drug exposure was also important. We counted with how many of 4 drug categories men were familiar before Vietnam -- marijuana, barbiturates, amphetamines, and opiates (usually codeine). All of those who had tried all 4 types before Vietnam used narcotics in Vietnam. The fewer drug categories with which men were familiar at arrival, the less the likelihood of trying narcotics in Vietnam. Nevertheless, one out of every 4 men who came to Vietnam with no drug experience whatsoever tried narcotics while there.

Use and addiction after return

In the first year after return, only 5% of those who had been addicted in Vietnam were addicted. The rate for men with urines positive at the time of departure was almost equally low. This finding was totally unlike the outcomes of young men treated at the Federal Narcotics Hospital in Lexington. When those young men were followed 6 months later, two-thirds were found already to be re-addicted (Stephens and Cottrell, 1972). The likelihood of any use, heavy use, and addiction for veterans was the mirror image of that for treated civilians. Nor was this good result transient. When we followed veterans at 3 years, only 12% of those addicted in Vietnam had been addicted at any time in the 3 years since return, and for those re-addicted, the addiction had usually been very brief. Their claim not to be still addicted was validated by finding few of them to have positive urines at either the first or the second follow-up interview.

It was not treatment that explained this remarkable rate of recovery. Only a third of the men addicted in Vietnam received even simple detoxification while in service, and only a tiny percentage of Vietnam enlisted men went to the Veterans Administration for drug abuse treatment after release from Service -- 4% of those who used narcotics in Vietnam, 6% of those who were positive at departure, and 14% of those positive at departure who continued to use after return. Yet, veterans who did enter treatment had relapse rates as high as the young civilian men in Lexington -- two-thirds had relapsed by the time we interviewed them (Figure 6). Relapse often occurred the very day they left the hospital.

Nor did recovery require abstention. Nearly half the men addicted in Vietnam tried narcotics again after return, but only 6% got re-addicted (Figure 7). Some were spared by using only narcotics other than heroin; some by not injecting, some by using only occasionally. But even regular heroin users became re-addicted in only half the cases.

We found few simple heroin addicts among the men who did become re-addicted on return from Vietnam. They also used a great variety of other drugs. More than 80% used amphetamines, more than 70% used barbiturates, and almost all used marijuana, and many had multiple dependencies. When heroin addicts were asked what their "main" drug was, more than half named alcohol or marijuana rather than heroin (Figure 8).

Nor did heroin seem uniquely associated with adverse social outcomes. Use of heroin on return was associated with more crime, unemployment, illegal employment, divorce or separation, violence, transiency, and credit problems than use of barbiturates or amphetamines, but not if we held constant pre-service behavior problems and the number of other types of drugs used (Figure 9). Indeed, the variety of drugs used was a better predictor of adverse consequences than which drugs were used.

Figure 6

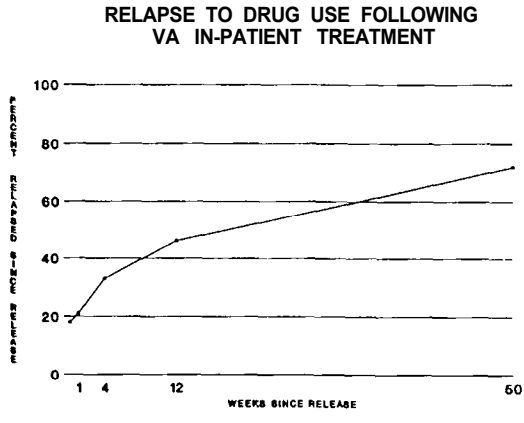


Figure 7

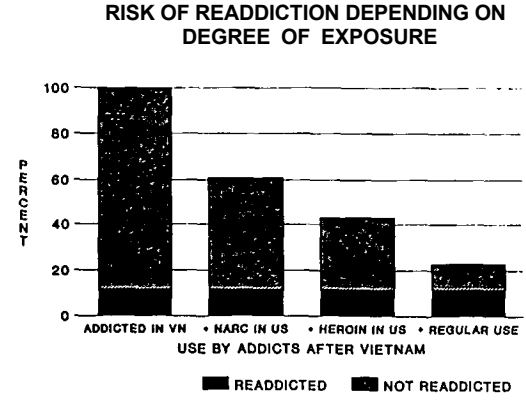


Figure 8

THE "MAIN" DRUG WHEN HEROIN AND OTHER SUBSTANCE USED AFTER RETURN FROM VIETNAM

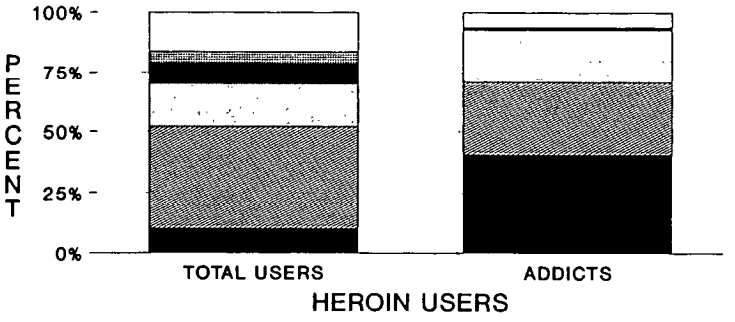
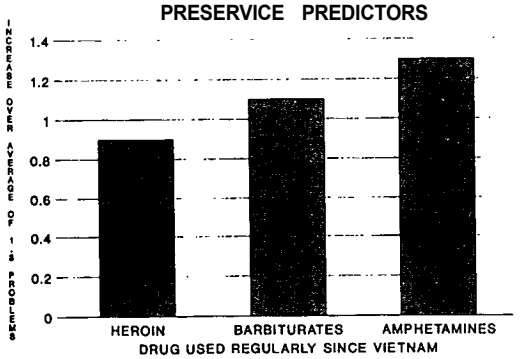


Figure 9

POST-VN DRUGS AND SOCIAL PROBLEMS, CONTROLS FOR NUMBER OF DRUGS AND PRESERVICE PREDICTORS



These then were the major conclusions: availability of a drug, rather than its chemical action or social consequences, appeared to determine its place in the hierarchy of popularity. When men moved to narcotics, they did not abandon the legal or illicit drugs they had used previously, but merely added narcotics to their repertoire. The most important determinants of narcotics use in Vietnam and after were pre-service behavior problems and drug experience. Narcotic addiction in Vietnam occurred in only about half those who used narcotics, and could be terminated voluntarily by most who became addicted. Re-addiction after return from Vietnam was rare and brief. Veterans re-addicted and entering treatment had as high a relapse rate as civilians. Heroin's adverse effects were no greater than those of amphetamines or barbiturates when juvenile behavior and concomitant use of other drugs was taken into account.

Reactions to the findings

The Department of Defense (DoD) was pleased with the study's findings, because they showed that Vietnam veterans had not been consigned to a life of unrelenting dependence on drugs, The press and the research community were more skeptical. They resisted giving up the beliefs that heroin was a uniquely dangerous drug, to which a user because addicted very quickly, and addiction to which was virtually incurable. They maintained these beliefs by offering 3 interpretations of our findings:

1) The results were false, perhaps tailored to exonerate the DoD.

2) Addiction in Vietnam was explained by the extraordinary setting -- the misery of war that made addiction a "normal" reaction; so the relatively benign outcome of addiction in Vietnam was irrelevant to addiction in the U.S.

3) The drop in frequency of addiction on return was caused by a change in setting. In the US. these men would not know where to get heroin, and the settings in which they lived and worked would not be associated in their minds with use or withdrawal symptoms, and therefore would not serve as stimuli to relapse. Thus again, the study findings were irrelevant to addiction beginning in the U.S.

I will argue that each of these views fails to fit the facts.

1) The idea of a whitewash by the DoD was extensively investigated by a New York Times reporter who had recently returned from that newspaper's Saigon office. During the 2 months' preparation of his article, he went over the study in great detail, often calling me to challenge what he took to be discrepancies between statements in my report. I spent a lot of time teaching him how to read graphs and tables, When the article finally appeared, it barely mentioned the Vietnam study. To get only one line in the two-page story that had been intended as an expose showed that the reporter could not support suspicions of a DoD whitewash.

2) The argument that addiction in Vietnam was a response to war stress, and therefore remitted on exit from the Vietnam war theatre, is still frequently cited as though it were self-evident, because it sounds so plausible. Yet this argument does not fit the facts. Heroin was so readily available in Vietnam that more than 80% were offered it. Those who became addicted had typically begun use early in their Vietnam tour, before they were exposed to combat. One man told us he was offered heroin as he got off the plane at arrival by a man who wanted to swap it for a clean urine so he could get onto that same plane to go home.

Veterans who saw active combat were more likely than others to use narcotics, but not once their pre-service histories were controlled for. Those with the pre-service antisocial behavior and drug experimentation that predicted drug use also saw more combat, presumably because their lack of school success and troubles with the law meant that they acquired none of the skills that kept cooks, typists, and construction workers behind the lines.

When we asked men why they used heroin, they did not tell us that they were trying to overcome fear or stress. Rather, they said it was enjoyable and made life in service bearable (Table 1).

3) The argument that men addicted in Vietnam would not relapse after return because they would not be re-exposed in the U.S. to the stimuli which had become associated with their drug use and withdrawal symptoms in Vietnam makes sense for those who did not become re-addicted in the U.S. However, it fails to account for the high rate of recovery while still in Vietnam. Nor does it explain why the periods of re-addiction for the minority who became re-addicted after return were so brief, since they remained in the setting in which the re-addiction occurred.

Table 1

WHY MEN USED NARCOTICS IN VIETNAM

REASON VOLUNTEERED	PERCENT
TO FEEL HIGH	40
TOLERATE ARMY REGULATIONS	13
RELIEVE BOREDOM	9
RELIEVE DEPRESSION	9
RELIEVE FEAR	8
PASS TIME	5
BE ONE OF THE GROUP	3

The findings of the Vietnam study cannot be dismissed by these claims of whitewash, extraordinary wartime stress, or change in setting. Yet mysteries remain. While we have powerful predictors of who would use drugs in Vietnam, and who would return to drugs when they got back to the States, none of our predictors told us anything about which of the veterans addicted in Vietnam who returned to heroin after return would get re-addicted (Table 2).

Nor can we be sure how generalizable these findings are. They are difficult to replicate in a sample of the population because heroin use is so rare -- only 1% have ever used it according to the Epidemiologic Catchment Area study (ECA) -- that studying heroin addiction in the general population is not practical.

Table 2

VARIABLES THAT FAILED TO PREDICT READDICTION
AMONG VETERAN ADDICTS WHO RETURNED TO NARCOTICS

BEHAVIOR PROBLEMS BEFORE SERVICE
PARENTS' SUBSTANCE ABUSE, ARREST, PSYCHIATRIC CARE
SUBSTANCE USE IN VIETNAM (TYPES, DURATION, INJECTION)
IQ
DRAFTEE OR VOLUNTEER
COMBAT
MILITARY DISCIPLINE PROBLEMS
DEMOGRAPHIC CHARACTERISTICS

THE ECA

In the 1980s. I was the principal investigator for one of 5 ECA sites. Heroin users were not many, even in this sample of 20,000. However, we were able to learn a little from that study. Unlike interviews in other ECA sites, the St. Louis interviews asked whether those who had ever met criteria for each type of substance abuse had experienced problems with it in the year before interview. We found that only 20% of those ever abusing opiates had had any symptom in the last year, a rate slightly lower than that for other drug types. This is a confirmation that heroin addiction is not interminable, and indeed tends to last no longer than addiction to other drugs.

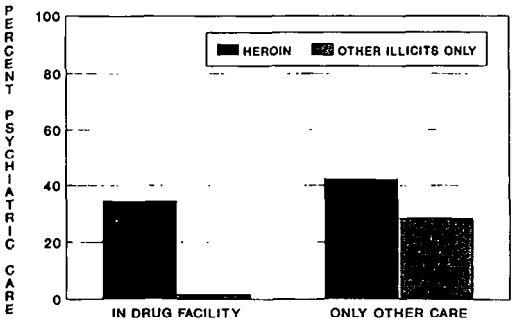
We also showed that heroin users, like abusers of all drugs except cannabis, usually abused a variety of drugs. We found virtually no one dependent only on narcotics, confirming the polydrug use of the Vietnam veterans.

Yet heroin continues to be regarded as a unique drug. We see the impact of these beliefs when we look at treatment experience. In the St. Louis ECA site, the average rate of receiving treatment in a special drug treatment facility in the 6 months before interview was 16% if there had been symptoms of drug abuse in the last year. If

symptoms were related to heroin, the treatment rate doubled, to 35%, more than 3 times the rate for abusers of any other drug (Figure 10). There are special treatment slots for heroin users because of public concern about it and because a unique treatment, methadone maintenance, is available to treat it.

Figure 10

TREATMENT THIS YEAR: HEROIN VS. OTHER ILLICIT DRUGS



ECA SAMPLE WITH DRUG PROBLEM THIS YEAR

In all ECA sites the importance of childhood conduct problems as a predictor of substance abuse was confirmed (Robins and McEvoy, 1990). To overcome the problem of the rarity of heroin abuse, we divided the sample according to the number of categories of substances with which problems had been experienced: alcohol, marijuana, opiates or cocaine, and all other illicit drugs. If there were problems with substances in 3 or 4 of these categories, sample members were considered to have serious substance abuse problems; if in only 1 or 2, they were considered to have mild problems. The more childhood behavior problems (e.g., lying, stealing, truancy, fighting, vandalism), the greater the likelihood of both severe substance abuse problems and any substance abuse problems (Table 3).

The ECA also allowed us to learn more about how conduct problems affected substance abuse. Conduct problems lowered the age at which substance use began. As the number of conduct problems increased, the age first drunk dropped from 17 years to 12; the age at first use of an illicit drug dropped from 21 years to 15. And the earlier use began, the more likely was the user to develop problems (Robins and Przybeck, 1985). But reducing age at first use was only part of the explanation for the impact of conduct problems on substance abuse. Even among those with a "normal" age of onset (i.e., ages 15 to 19). a history of prior conduct problems was associated with increased risk of problems (Figure 11). Development of substance

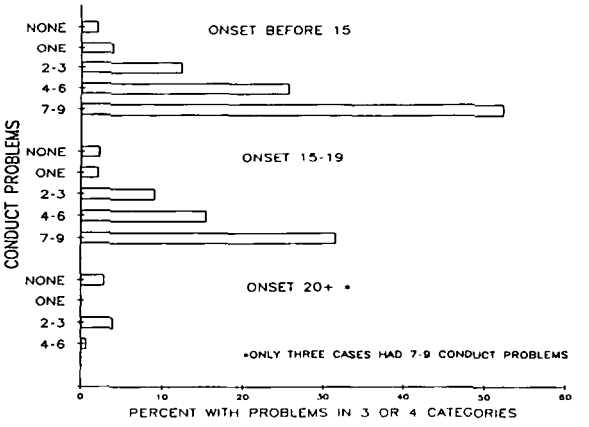
Table 3

RANGE OF CONDUCT PROBLEMS BEFORE 15
AND SUBSTANCE ABUSE PROBLEMS AMONG USERS

Number Of		Categories of Substances With Problems			
		None	1-2	3-4	Total
Conduct Problems	N	%	%	%	%
None	1021	62	35	2	100
One	1096	48	50	2	100
Two	1006	35	57	8	100
Three	746	26	61	13	100
Four	544	20	65	15	100
Five	438	17	62	21	100
Six	331	11	61	28	100
Seven	207	4	54	42	100
Eight	143	2	42	56	100
Nine	49	0	46	54	100

Figure 11

CONDUCT DISORDER AND SUBSTANCE ABUSE
CONTROLLING ON AGE OF FIRST USE



abuse problems appears particularly enhanced when there is both early exposure and a history of prior behavior problems. Nonetheless, substance abuse is not just a part of conduct disorder, A substantial minority of substance abusers have no history of prior conduct problems, although they often develop a later history that looks much like that predicted by early conduct difficulties.

LINKING EPIDEMIOLOGY TO LABORATORY SCIENCE

It seems to me that the important message that comes out of these epidemiological studies of substance abuse is that there is enormous variation in how human beings respond to potentially addictive substances. Some of these differences appear to be the effects of prior experiences, both with other substances and with other behaviors that seem to affect later drug responses. Yet there are clearly limits to how much of this diversity epidemiologic research can explain. Can laboratory researchers make use of epidemiologic observations and design studies that can explain individual variation, as they have previously endeavored to explain variation across drugs and across species?

For example, we wonder why age of first substance use makes such a difference in outcome. Is it because the brain does not complete its maturation until late adolescence and these substances do permanent damage only to immature brains? Age of exposure appears to interact with prior conduct problems. Could one model this combination in the laboratory by comparing long-term effects of drug exposure in aggressive, immature animals to effects in older aggressive animals and immature non-aggressive ones?

Human beings who use heroin or cocaine have typically used other types of drugs earlier, and continue to use them along with the heroin or cocaine. Can laboratory studies examine the transfer of experience across drug types, and the effects of concurrent use of multiple drug types? Laboratory studies would allow studying the interactions among drugs without the confounders of adverse social consequences that so confuse the picture for human beings.

When I first sat on the Committee on Problems of Drug Dependence in the early 1970s some social scientists were claiming that the euphoric effects of drugs require that the user expect them. Animal experiments have since convinced all but the diehards among us that the rewards of drug taking depend on biological action in the brain as well as on socialization by the peer group. I hope that the time is approaching when social scientists can return the favor by providing insights that will be as useful to laboratory science as laboratory science has been to social science. Discovering biological mechanisms that explain the diversity of human reactions to drugs has obvious potential for selecting those in need of preventive strategies, and may suggest treatments to increase resistance to drug effects. Epidemiologists have found no social variables that predict the transition from heavy use to addiction. Nor do we understand why almost all drug use wanes and disappears in middle age. Perhaps these transitions are almost entirely biologically determined. Perhaps noting where epidemiology has not shed light on mechanisms of abuse and dependence will suggest critical laboratory experiments that will further understanding how biology affects drug behavior.

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OPIOID RECEPTORS: MOLECULAR AND STRUCTURAL STUDIES

E. J. Simon

INTRODUCTION

This was a very exciting symposium, in which we heard the first oral presentations on the successful cloning of an opioid receptor, the δ receptor from NG108-15 cells. This was achieved simultaneously by the laboratories of Drs. C. Evans and B. Kieffer, both of whom presented their results. Probes from the δ receptor cDNA were subsequently used to screen for other types of opioid receptors. By now all three major types, μ δ, and κ, have been cloned and sequenced. Drs. L. Yu and G. Uhl, who simultaneously cloned the μ receptor were added as discussants in the last minute. Dr. T. Reisine whose laboratory was successful in the cloning of a κ receptor was not available.

New data on the reconstitution and characterization of a highly purified uI receptor were presented by Dr. J. Hiller and novel studies on second messenger pathways of opioid receptors were reported by Drs. P. Law and S. Childers. A brief summary of each talk follows.

PROGRESS IN THE CLONING AND SEQUENCING OF THE cDNA OF THE DELTA OPIOID RECEPTOR FROM NG108-15 CELLS.

Christopher J. Evans, UCLA, Los Angeles, CA.

Our strategy for cloning of a delta opioid receptor from NG108-15 cells was as follows: a random-primed cDNA expression library was constructed in the expression vector CDM8 using mRNA isolated from NG108-15 cells. COS cells were transfected with the library and receptor expressing cells identified by screening COS cell lawns with the opioid peptide ligand DALA^{2}DLeu^5-enkephalin radiolabeled with ^{125}I at the N-terminal tyrosine. Positive cells were detected by autoradiography and plasmids isolated from these cells were amplified in bacteria then transfected into COS cells. Following three rounds of screening a pure clone, DOR-1, was identified and fully characterized.

The receptor protein is 372 amino acid in length. There are two consensus N-linked glycosylation sites close to the N-terminus and a third consensus glycosylation site is present at residue 352, though this site resides in the C-terminal domain of the protein which is predicted to be intracellular. The sequence of DOR-1 contains many consensus protein kinase C as well as cAMP or cGMP dependent kinase sites. Interestingly, there is a cluster of kinase sites in the third intracellular loop, which, by analogy to other G-protein-coupled receptors, may be critical for interaction with G-proteins. It is anticipated that phosphorylation of the receptor will be involved in the adaptive responses to continued exposure to agonists as has been elegantly demonstrated for the ß-adrenergic receptor. By analogy with other receptors Cys^{333} is postulated to be a potential palmitoylation site.

Of particular note are aspartate residues at positions 95 (conserved in all somatostatin receptors) and 128 in the second and third proposed membrane spanning regions. Equivalent aspartic residues in the ß-adrenergic receptor are implicated in ligand binding. For binding of both peptides and alkaloids to opioid receptors there is an absolute requirement for a positive charge and it appears likely that Asp 128 in the third membrane spanning region of DOR-1 could serve as the counterion for opioid binding.

Homology comparisons with other G-protein coupled receptors have revealed high homology with receptors for somatostatin, angiotensin, 118 and n-formyl peptide. The high homology of DOR-l with somatostatin receptors is of particular interest. Presently there are four isoforms of somatostatin receptors that have been identified and DOR-1 has highest homology with isoform 1. In addition to binding to a variety of somatostatin receptors, somatostatin binds to mu opioid receptors, albeit with low affinity. Analogs of somatostatin with high affinity for mu receptors have recently been synthesized and demonstrated to be opioid antagonists. As is the case for opioid receptors, stimulation of somatostatin receptors results in inhibition of neurotransmitter release by inhibiting Ca^{2+} currents and increasing K^{+} conductance.

Localization of delta receptor mRNA was studied by in situ hybridization. Similar hybridization patterns were observed in both mouse and rat brains, although more intense labeling was detected in the mouse brain sections, consistent with the murine origin of DOR-1. The most striking feature of the neuroanatomical distribution of DOR-1 mRNA is the intense labeling in the olfactory bulb, predominantly localized to the external plexiform layer. Additional hybridization signals in olfactory structures were found in the olfactory tubercle and anterior olfactory area. Diffuse labeling is present throughout the cortex and basal ganglia, the caudate putamen, hybridizing more intensely in lateral than medial regions. Other limbic areas such as amygdala, hypothalamus and hippocampus also show hybridization. In the brainstem the pontine and interpeduncular nucleus show strong labeling and more diffuse and less intense hybridization is observed in the substantia gelatinosa of the cervical spinal chord. No detectable hybridization is present in the thalamic nuclei and mouse cerebellum, although there is some labeling of deep cerebellar nuclei in the rat. These observations parallel the distribution of delta opioid binding as determined by receptor autoradiography with exception of the rat cerebellum and the strong hybridization in the pontine nucleus. All the labeling could be competed by the incorporation of a 50x excess of unlabeled probe in the hybridization mixture.

Finally we report on DOR-1 mRNA in mouse brain and NG1OB-I5 cells. Preliminary analysis of the multiple transcripts in NGl08-I5 cells indicate that the two smaller bands (2.2 and 2.5KB) appear to result from alternative polyadenylation sites. Mouse brain has only two major transcripts at about 8.5 and 10KB. With regard to regulation of DOR-1 transcripts, little change in DOR-1 mRNA levels is revealed after treatment with DADLE or etorphine. However, treatment of the cells with forskolin and IBMX caused a dramatic down regulation of DOR-1 mRNA levels.

THE MOLECULAR CHARACTERIZATION OF THE DELTA OPIOID RECEPTOR BY EXPRESSION CLONING

Brigitte L. Kieffer, Ecole Superieure de Biotechnologie, Strasbourg, France

Opioid receptors are membrane proteins which mediate the analgesic effect of opium-derived alkaloids. Progress in the design of potent analgesics, devoid of severe side-effects, was strongly limited by the lack of knowledge of the primary amino acid sequence and the secondary and tertiary structure of the receptors.
In order to achieve the molecular cloning of one of these receptors, we constructed a random-primed expression cDNA library from NGl08-15 cells. These cells are known to express opioid receptors which are exclusively of the delta type. Pools of plasmid DNA were transfected into COS sells which were screened for the ability to bind tritiated Tyr-dAla-Gly-Phe-Leu-Thr (DTLET). A cDNA was isolated which encodes a membrane protein with all the structural characteristics of a G-protein coupled receptor. Analysis of the deduced protein sequence indicates that this receptor is most probably entirely embedded in the membrane. Its amino acid sequence is highly homologous to that of the somatostatin receptors with which it shares an interesting feature: the presence in the same receptor of both the short cytoplasmic loop typical of peptide-binding G-protein-coupled receptors and the conserved Asp residue in the third transmembrane domain, characteristic of receptors that bind amine neurotransmitters, such as catecholamines and acetylcholine.

When expressed in COS cells, the receptor exhibits pharmacological properties typical of a delta opioid receptor: high affinity binding sites for DTLET (Kd=1.4nM)), stereoselectivity and a highly selective pharmacological profile, characteristic of delta receptors. The cloned receptor transiently expressed in COS cells is negatively coupled to adenylyl cyclase, i.e., intracellular cAMP accumulation is decreased by opioids in a concentration-dependent manner. These data indicate that the cDNA we have isolated encodes the delta opioid receptor present in NG108-15 cells. Northern analysis studies show that this receptor is also expressed in mouse tissues. The major transcript is 8.5kb in size and is found in brain. It is also weakly present in heart but not detectable in kidney, lung, liver or spleen.

The availability of this cDNA should allow the isolation of clones encoding other opioid receptor types and subtypes. The characterization of all members of the opioid receptor family will ultimately lead to a better understanding of their involvement in nociception and analgesia.

RECENT STUDIES ON THE STRUCTURE AND RECONSTITUTION OF PURIFIED MU OPIOID RECEPTORS

Jacob M. Hiller, New York University Medical Center, New York, NY.

An opioid binding protein (OBP), purified to homogeneity from bovine striatal membranes, has been reconstituted into liposomes. For most experiments the necessary lipids and G-proteins were provided by a CHAPS extract of bovine striatum. This extract was prepared in such a way that it was devoid of opioid binding activity. The liposomes were produced by precipitation of a mixture of OBP and CHAPS extract with polyethylene glycol 6000 and resuspension in buffer containing calcium but devoid of sodium. While soluble OBP binds opioid antagonists with high affinity it binds opioid agonists with low affinity, characteristic of a receptor uncoupled from G-protein. However, reconstituted OPB bound the mu opioid agonist, [^{3}H]DAGO, saturably and with high affinity, equivalent to that seen in membrane-bound mu-opioid receptors (1.5 nM). Competition binding studies against the universal opioid ligand [^{3}H]bremazocine with mu, delta and kappa agonist ligands demonstrated the high mu selectivity of the reconstituted OBP. Stereoselectivity of binding was shown by the inability of (+) naloxone to compete gainst 3H-DAGO, while (-) naloxone competes with a K_j of 2nM. We have also found that GTPγS completely inhibits [^{3}H]DAGO binding to the reconstituted OBP, confirming the successful coupling between receptor and G-protein. The stimulation of low K_m-GTPase by DAGO has also been demonstrated. In preliminary experiments, carried out in liposomes made by PEG precipitation and resuspension of a mixture of OBP, bovine brain phospholipids (Sigma) and purified G-protein, G_j or G_{oa}, (courtesy of Drs. J. Hildebrand, J. Dinkus and M. Wilcox), high affinity [^{3}H]DAGO binding was restored.

Peptide fragments of OBP, generated by chemical cleavage, have been microsequenced and polyclonal antibodies against synthesized segments of the peptides, conjugated to thyroglobulin, have been produced in rabbits. Antibody 165, which recognizes the unique amino acid sequence, IRNLRQDRSKYY, was used in an immunohistochemical study in rat brain. The distribution of immunoreactive perikarya and neuronal processes in various regions of the brain and spinal cord correlate well with mu-ligand binding distribution as revealed by autoradiographic techniques.

ANALYSIS OF DELTA OPIOID RECEPTOR FUNCTIONS

P.Y. Law amd H.H. Loh, University of Minnesota Medical Center, Minneapolis, MN.

In neuroblastoma x glioma NGl08-15 cells, activation of delta-opioid receptors results in multiple cellular responses. Delta-opioid agonists lower the intracellular cAMP level by inhibiting adenylyl acyclase activity and stimulating phosphodiesterase activity. They regulate intracellular calcium levels by regulating voltage-dependent calcium channels and by controlling phospholipase C activity, and thereby IP3 level. This coupling to multiple

effectors could be due to the presence of multiple delta-opioid receptors in NG108-15 cells, the ability of a homogeneous population of delta-opioid receptors to interact with multiple G-proteins, or the presence of one or more subtypes of delta opioid receptors able to interact with a single G-protein, which is able to regulate multiple effectors. It is the purpose of current studies to distinguish the above possibilities. We examined the identity of G-proteins which interact with delta-opioid receptors in NG108-15 cells, By taking advantage of the ability of agonists to promote GDP dissociation and GTP association to the G_{α}-subunit of heterotrimeric G-proteins, and using the photoaffinity labeling analogue of GTP, [^{32}P]GTPazidoanalide, we were able to demonstrate DADLE-concentration-dependent radioactive labeling of proteins with molecular mass 39-41 kDa. By separating these proteins by urea-SDS-PAGE and identification of the proteins by Western analysis with G_{α}-subunit specific antibodies, we demonstrated that DADLE promoted [^{32}P]GTPazidoanilide labeling of $Gi_{2\alpha}$, both isoforms of G_{oa} and the isoform, of G_{i3a} found in NG108-15. Furthermore, similar concentrations of DADLE were required to promote the association and hence labeling of the G_{α}-subunits by GTPazidoanilide. One possibility was that multiple delta-opioid receptor subtypes are being expressed at a high level in NG108-15 cells. We therefore stably transfected Chinese hamster ovary cells (CHO) with a recently cloned delta-opioid receptor cDNA (courtesy C. Evans). Different levels of receptor expression were obtained in several clones of CHO. Although the amount of [^{32}]GTPazidoanilide incorporation by G_{α}-subunits was dependent on receptor concentration, DADLE promoted [^{32}P]GTPazidoanilide labeling of $G_{i2\alpha}$, G_{oa} and $G_{i3\alpha}$ in all three clones of delta-opioid receptor transfected CHO cells tested. The ability of delta-opioid receptors to couple to other G-proteins was demonstrated further when in vitro transcribed delta-opioid receptor mRNA was co-injected into *Xenopus* oocytes with CFTR, a chloride channel which can be activated by elevated intracellular cAMP levels. Instead of the predicted lowering of intracellular cAMP, application of DADLE to oocytes injected with delta receptor and CFTR mRNA's resulted in an increase in Cl-current, indicative of an elevation of intracellular cAMP level, which could be blocked by naloxone. A similar increase in Cl-current was observed when isoproterenol was applied to oocytes which had been injected with ß-adrenergic receptor and CFTR mRNAs. It is postulated,that in Xenopus oocytes, which do not express $G_{i2\alpha}$, delta-opioid receptors regulate intracellular cAMP levels by coupling to $G_{s\alpha}$. How delta-opioid receptors regulate different effector systems when they are able to couple to multiple G-proteins remains unsolved.

SIGNAL TRANSDUCTION MECHANISMS FOR OPIOID RECEPTORS

Steven R. Childers, Department of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC.

The recent cloning of the delta opioid receptor by Evans et al. and Kieffer et al. have confirmed the G-protein-coupled nature of this receptor. The deduced structure contains seven transmembrane segments and other features which place it in the G-protein superfamily of receptors. Such a structure was predicted some years ago from the finding that the three major types of opioid receptors (mu, delta and kappa) all produced responses typical of receptors which belong to the $G_{i/o}$-coupled family. These responses can be detected throughout the signal transduction pathway, including the following components:

1. Receptor. Guanine nucleotides inhibit the binding of most agonists to receptor sites by increasing their dissociation rates. In this way, the high affinity state of opioid receptors for agonists is eliminated by GTP and sodium. The recent development of agonists which are insensitive to this effect dramatically increases their potencies under conditions where high affinity agonist binding is normally lost.

2. G-protein. Significant progress has recently been made in determining the identity of specific G-proteins coupled to opioid receptors. These G-proteins include several subtypes of G_i and G_o. The precise G-protein coupled to different opioid receptors may depend on the cell type. Moreover, opioid agonists stimulate low K_m GTPase present on the alpha subunit of the G-protein. This activity is a measure of agonist efficacy, as well as a

mechanism for inactivating G-protein. Methods which decrease GTPase activity (such as treatment at low pH) thus increase agonist efficacy.

3. Effectors. All opioid receptor types inhibit adenylyl cyclase. They are also coupled to ion channels, particularly to the closing of Ca^{2+} and the opening of K^{+} channels. The mechanism of receptor action in most cases involves direct coupling of the receptor to the channels through the G-protein without an intervening diffusible second messenger system. However, other mechanisms may occur in other cell types, and other effectors may be identified in the future.

4. Post-effector events. Opioid-inhibition of adenylyl cyclase results in the inhibition of the phosphorylation of several proteins. Major opioid-inhibited phosphoproteins in brain are synapsins I and II, which are vesicular proteins that play a role in mediating neurotransmitter release. Reduction in synapsin phosphorylation is consistent with the actions of opioid agonists in inhibiting neurotransmitter release. Inhibition of intracellular cAMP also plays an important role in regulating several genomic events. For example, the proenkephalin gene contains a cAMP-responsive element which is stimulated by cyclic AMP-dependent protein kinase. Opioid agonists reduce proenkephalin mRNA synthesis by reducing cAMP levels, thus providing a potential negative feedback system for regulation of opioid peptide levels.

5. Regulation of signal transduction systems during opioid tolerance and dependence. Two different mechanisms are relevant to the role of signal transduction pathways in the development of opioid tolerance and dependence. The first event involves desensitization of receptor-effector coupling, followed by receptor downregulation. These events occur in cell culture, and are mediated by processes typical of other $G_{i/o}$-coupled receptors. However, in brain, desensitization of opioid-inhibited adenylyl cyclase does not commonly occur during chronic agonist treatment. In this tissue, a second process occurs which involves compensatory changes in signal transduction systems. In this process, activities which were inhibited by acute opioid action become stimulated during chronic agonist treatment. Thus, basal levels of adenylyl cyclase, activity of cyclic AMP-dependent protein kinase, and phosphorylation of synapsin are all increased during chronic morphine treatment. These changes occur in the direction opposite to those observed after acute morphine administration, and may contribute to the overall phonemena of tolerance and dependence.

THE CLONING OF THE MU OPIOID RECEPTOR

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When the chairpersons learned that the mu receptor had also been cloned, Drs. George Uhl and Lei Yu were added to the program as discussants. In brief, both laboratories made use of cDNA probes derived from the nucleotide sequence of the recently cloned delta receptor cDNA. In one case (Uhl), PCR was used with single stranded cDNA from whole rat brain as the primer to obtain a 700 base pair insert. Rat brain cDNA libraries were screened at low stringency. In both laboratories a clone was obtained which had a high degree of sequence homology with the cDNA of the murine delta receptor. The amino acid sequence exhibited the structural features of a G-protein coupled receptor. When transfected into COS cells, the cDNA conferred on these cells a binding site with the pharmacological characteristics of a mu opioid receptor. Yu and coworkers found that DAGO decreased the accumulation of cAMP and this decrease was reversed by naloxonazine, supporting the functional coupling of the mu receptor to G-protein. Uhl and coworkers found high levels of a 10.5 kB mRNA in thalamic neurons which hybridizes with the cDNA from their mu receptor clone.

Summarized by Eric J.Simon, New York Univ. Med. Ctr., New York, NY

THE TERATOGENICITY OF THE DRUGS OF ABUSE: A SYMPOSIUM

L. P. Finnegan, A. P. Streissguth, G. Koren, D. Neuspiel and K. Kaltenbach

A great deal of concern has been expressed over the last several decades concerning the effects of licit and illicit drugs upon pregnancy, the fetus, the newborn and child. Numerous research studies have undertaken the difficult task of determining the effects of alcohol, cocaine, and opiates from a physiological and developmental standpoint. Many issues have been clearlv defined with regard to the effects of alcohol on pregnancv. the fetus. and the newborn and its relationship to long-term development. Fetal Alcohol Syndrome has been described as well as Fetal Alcohol Effects. Long-term studies involving infants prenatally exposed to alcohol have been on-going over the last decade.

With regard to cocaine, despite a growing number of studies that have investigated the reproductive effects of maternal cocaine use, a homogeneous pattern of fetal effects has not been established, and, there is little consensus on the adverse effects of the drug. Although, considerable concern has been expressed about the high rate of cocaine use among pregnant women; a rush to judgement about the extent and permanency of specific effects of intrauterine cocaine exposure has occurred. Many predictions with regard to cocaine have been promulgated by the scientific and lay communities in spite of the lack of supportive scientific evidence. Many have been concerned about the potential severity and universality of cocaine effects, but premature conclusions may be potentially harmful to children.

Infants have been exposed to opiates for centuries and it has only been in the last two decades that researchers have generated a number of studies evaluating the short and long-term effects. The neonatal abstinence syndrome has been defined and recommendations for treatment have been published. However, studies on long-term effects are limited and also, as in the research of cocaine effects confounded by a host of variables seen in pregnant opiate-dependent mothers. This symposium presented evaluations of the current literature with regard to longitudinal outcomes of children exposed to alcohol, structural defects and behavioral outcomes of infants exposed to cocaine, and the long-term effects of opiate exposure in utero.

ALCOHOL: LONGITUDINAL OUTCOMES

The Seattle Longitudinal Prospective Study on Alcohol and Pregnancy involves around 500 children who have been examined at various points in time, including day 1 and 2 of life, 8 and 18 months, 4, 7, and 14 years, with data from classroom teachers obtained at 7 and 11 years. Follow-up of the cohort has been maintained at around 82 percent of the original cohort, with no systematic loss of high-risk subjects. Mothers were primarily white, middle class, well-educated married women at fairly low risk for adverse pregnancy outcome. All were in prenatal care by the fifth month of pregnancy, when they were interviewed regarding drinking habits and other potential covariates. Approximately 80 percent were drinking during pregnancy, with the same proportion drinking prior to pregnancy.

A recent longitudinal analysis of the data for the first seven years of life involves analysis by the Partial Least Squares, a relatively new multivariate statistical method combining themes from factor analysis, multiple regression and non-linear scaling. Our principal findings are as follows: (1) Effects of prenatal alcohol exposure am manifest at all ages from birth to seven years; they arc manifest on a variety of behavioral measures; and they do not attenuate

with time. (2) Among the most salient sequelae of exposure. are neonatal habituation to light, time in error from the Wisconsin Motor Steadiness Battery at age four, standardized WISC-R and WRAT-R arithmetic subtests at age seven years. and academic adjustment as rated by the second grade teacher. (3) For most of the outcomes, binge drinking has more serious consequences than the same amount of steady drinking, and drinking early in pregnancy has more serious consequences than the same reported pattern of drinking in mid-pregnancy. (4) There is no statistical evidence for a "risk-free" threshold level of prenatal drinking. (5) These alcohol effects cannot be "explained away" by any of 150 covariates we considered; including Parents' education. prenatal nutrition, and prenatal exposure to nicotine. (6) Profiles of alcohol-related Scholastic and neurobehavioral deficit strikingly independent of the usual covariates are manifest by the second grade in school. These show promise for the characterization of individual children as fetal-alcohol-affected based on neurobehavioral criteria.

Data from classroom teachers when the children were 11 years old revealed prenatal alcohol-related difficulties with classroom behavior, academic performance (particularly arithmetic), and information processing. At 14 years of age, continued effects of prenatal alcohol on laboratory measures of attention and memory were observed, as well as problems with phonological processing and numerical reasoning.

COCAINE: STRUCTURAL DEFECTS

Over the past decade, a large number of studies addressed the association between in *utero* cocaine exposure and malformation rates. Invariably, these studies have compared rates of malformation in cocaine-exposed vs. cocaine-unexposed babies. There are serious methodological issues with these studies. Firstly. exposure is often verified by maternal history, which is less than 50 percent sensitive; urine tests become negative within a few days due to the short elimination half-life of the drug. Secondly, mothers who use cocaine have clustering of other risk factors, including alcohol and cigarette consumption, poor prenatal care, sexually transmitted diseases, poor nutrition and low SES. Therefore, association of the fetal adverse effects does not prove causation.

In meta-analysis of all published papers, we have revealed that for most end points, positive studies are balanced by negative studies. When cocaine-exposed children are compared to middle-class women and their children, the former suffer from many more adverse effects. However, when they are compared to babies exposed to other drugs of abuse but no cocaine, most adverse effects are nullified.

In summary, while cocaine does not appear to be a major human teratogen, we hypothesize that polymorphic metabolism and placental transfer may expose some babies to its potent pharmacological effects.

COCAINE: BEHAVIORAL OUTCOMES

The behavioral effects of intrauterine cocaine exposure on the infant and child are controversial, with various studies reporting conflicting results. Inconsistent findings may emerge from various methodological problems in this research, including sampling, exposure measurement, cohort retention and selection of dependent variables. The problem of confounding is of particular concern. To be a confounder, a variable must be: a) a risk factor for the study outcome, regardless of cocaine exposure status; b) associated with cocaine exposure; and c) not be an intermediate factor in the causal pathway between cocaine exposure and outcome. In the presence of a strong confounder and weak or non-existent primary exposure effect, misclassification of confounding may lead to biased results.

Extant studies of cocaine effects on infant and child behavior have not consistently or accurately assessed confounding effects. For example, tobacco smoke exposure has been considered in most of these studies, its measurements have been limited, with potential for misclassification and undercontrol for smoking effects. Many other confounders have been inadequately controlled in these investigations. The effects of undercontrol for confounding may lead to greater positive bias (falsely reporting adverse outcomes from cocaine exposure) than the negative bias resulting from misclassification of cocaine users as non-users.

These methodologic problems may lead to biased magnified estimates of the risk of cocaine exposure. This may have clinical and policy consequences, including unnecessary termination of pregnancy, labeling and reduced expectations for cocaine exposed children, and decreased attention to social programs directed toward other sources of behavioral risk. Attention to improved methodology in future research can result in a clearer view of the true risks of cocaine exposure to the behavior and development of infants and children.

OPIATE EXPOSURE

An overview of existing research on prenatal opiate exposure, identifying limitations of past work and offering suggestions for future research paradigms was presented. A critique of current research investigating the effects of prenatal cocaine exposure leaves one with a sense of *deja vu.* Opiate research in the late 70s and early 80s typically used a bi-variate approach with little or no attention to multiple confounding factors. While the signs and symptoms of neonatal abstinence were well documented, findings among studies were often diverse as women differed on whether or not they were maintained on methadone; daily methadone dose; length of methadone maintenance during pregnancy; and amount of prenatal care. Additionally, poly-drug abuse including alcohol and nicotine use was rarely taken into account. By the mid-80s most investigators recognized that not only biological but social and environmental risk factors must be considered and began to call for a multi-factorial approach to investigate perinatal and developmental outcomes associated with prenatal opiate exposure. Unfortunately, as research strategies evolved toward a multi-variate approach, funding interest in the effects of opiates *(i.e.,* methadone) began to wane and the cocaine epidemic produced a wave of investigators naive in the field of maternal addiction and prenatal drug exposure. Returning to the critique of research investigating the effects of prenatal cocaine exposure suggests that we have come full circle. Investigators are again beginning to call for a multi-factorial approach. Aside from issues concerning the strategies to control for confounding effects, the need to address prenatal drug exposure within a multi-risk model in order to identify the effects of cumulative risks concomitant to maternal substance abuse is most relevant to the accomplishment of this research.

In summary, research studies on prenatal exposure to alcohol, cocaine, and opiates have defined a number of structural and behavioral effects upon the fetus and newborn. It is clear, from a pharmacological basis and animal research, that many of these effects are biologically plausible through direct or indirect mechanisms. However, the research to date has not clearly defined specific independent effects, such as in the case of cocaine in human infants. In reviewing previous studies on long-term effects (to school age), except in the case of alcohol, few studies have been reported with regard to opiate exposure, and no studies have evaluated infants exposed to cocaine. Research in the area of prenatal exposure to licit and illicit drugs is of key public health importance. Moreover, researchers and funding agencies must work diligently to advance the research from the methodological weaknesses of the past and to define the risk and nature of structural as well as behavioral outcomes. While defining these effects, researchers, clinicians, and those involved in public policy should clearly develop intervention services for both mothers and children and to advocate for improved health care and drug treatment services for those women who are dependent upon alcohol, cocaine, or opiates.

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CANNABINOID RECEPTORS: PHARMACOLOGY, SECOND MESSENGER SYSTEMS AND ENDOGENOUS LIGANDS

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Research during the past few years has dramatically expanded our knowledge of the actions of cannabinoids in the brain. Prior to this time, it seemed that one could invoke an argument for almost any neurochemical system as a cannabinoid substrate. We now know that there are specific cannabinoid receptors discretely located throughout the brain which are G-protein coupled. An endogenous substance has been isolated from brain which binds to this receptor and produces cannabinoid effects. The purpose of this symposium was to summarize these latest discoveries.

CANNABINOID TOLERANCE STUDIES WITH THE MOUSE VAS DEFERENS

One of the primary goals of Dr. Pertwec's laboratory is to explore the suitability of the mouse isolated vas deferens as a model with which to study the basis of cannabinoid tolerance. The experiments described here were directed at establishing whether *in vivo* pretreatment with Δ^9-tetrahydrocannabinol (THC) can induce tolerance to the inhibitory effects of psychotropic cannabimimetic agents on electrically-evoked contractions of the mouse isolated vas deferens. Previous experiments in this laboratory had shown that tolerance to THC-induced hypothermia can develop rapidly in mice and it was therefore decided to determine whether a THC pretreatment producing such tolerance would also induce cannabinoid tolerance in the vas deferens. Apart from THC, the drugs used in this investigation were CP-55,940, WIN 55,212-2 and the putative endogenous cannabinoid, anandamide. These drugs bind avidly to cannabinoid binding sites, possess cannabimimetic pharmacological properties, and yet have markedly different chemical structures both from each other and from THC.

Male MFl mice were injected i.p. once daily for two days with either THC (20 mg/kg) or Tween 80 (40 mg/kg). The hypothermic effects of THC, CP-55,940, WIN 55.212-2 and anandamide were measured 24 h after the second i.p. injection of THC or Tween by noting the maximum decrease in rectal temperature produced by each compound. Unless stated otherwise, these compounds were injected intravenously (n=6). In other experiments, mice were killed 24 h after the second pretreatment with THC or Tween 80 and their vasa deferentia removed and placed in an organ bath in order to determine the effects of the cannabinoids on electrically evoked contractions. THC (1.0 mg/kg, i.v.) was significantly less hypothermic in THC-pretreated mice (0.19 ± 0.13 °C; means ± s.e.) than in Tween-pretreated mice (2.77 ± 0.43 °C; $P < 0.001$). Similarly, THC (10 nM) inhibited the twitch response to a lesser extent ($P < 0.001$) in vasa deferentia obtained from THC-pretreated mice (5.4 ± 5.1%; n=8) than in tissues obtained from Tween-pretreated animals (42.4 ± 5.5%; n=8). The inhibitory effects of CP-55,940 (0.316 nM), WIN 55.212-2 (3.16 nM) and anandamide (100 nM) on the twitch response were also significantly less ($P < 0.05$) in tissue from THC-pretreated mice (13.6 ± 3.8%. 23.2 ± 4.9% and 27.4 ± 4.7%. respectively; n=5 to 8) than in tissue from animals pretreated with Tween (40.2 ± 4.9%, 77.16 ± 4.4%. and 49.5 ± 8.9%, respectively; n=6 to 8). Results from other experiments indicated that the degree of tolerance produced, both to the hypothermic effect of THC and to its inhibitory effect on the vas deferens, was directly related to the size of the pretreatment dose of THC and to the number of ptetreatments given (data not shown). Additional experiments were carried out to establish whether the *in* vivo pretreatment with THC that had been found to render the vas deferens tolerant to CP-55,940, WIN 55,212- and anandamide would also induce tolerance to the hypothermic effect of these drugs. It was found that CP-55,940 (0.05 mg/kg) and WIN-55,212-2 (0.2 mg/kg) were indeed significantly less hypothermic ($P < 0.001$) in THC-pretreated mice (0.05 ± 0.20 °C and 0.08 ± 0.13 °C

respectively) than in animals that had been pretreated with Tween (2.07 ± 0.31 C and 2.16 ± 0.31 °C respectively) whereas anandamide (10 mg/kg) was no less hypothermic in THC-pretreated mice (1.32 ± 0.22 C) than in Tween-pretreated animals (1.47 ± 0.07 °C; P > 0.05). Hypothermic responses of THC-pretreated and Tween-pretreated mice to anandamide (20 mg/kg, i.p.) were also found not to differ significantly from each other (2.54 ± 0.27 °C and 2.22 ± 0.22 °C respectively; n=5).

These results confirm that cannabinoid tolerance can be rapid in onset and provide further evidence for the hypothesis that is mainly pharmacodynamic in nature. Our finding that *in vivo* pretreatment with THC can induce tolerance both to its own inhibitory effect on the mouse isolated vas deferens and to that of other cannabimimetic agents suggests that this preparation would be suitable as an experimental model for investigating the mechanisms responsible for cannabinoid tolerance.

CANNABINOID RECEPTOR-MEDIATED G-PROTEIN COUPLED ACTIVITIES IN CEREBELLUM AND HIPPOCAMPUS

The discovery that Δ^9-THC and other cannabinoids bind to G-protein-coupled receptors (Howlett, 1985) is a discovery with profound implications for the molecular and cellular mechanisms of these drugs in brain. The cannabinoid receptor is a member of the $G_{i/o}$ family of receptors, containing a single protein subunit with seven transmembrane segments (Matsuda *et al.,* 1990). Cannabinoid agonists inhibit adenylyl cyclase through pertussis toxin-sensitive G-proteins, and they act directly through G-proteins to close calcium channels (Mackie and Hille, 1992). The research presented by Dr. Childers describes the properties of cannabinoid receptors in brain membranes and cerebellar granule cells, explores the relationship between cannabinoid receptors and other G-protein-coupled receptors, and characterizes the possible role of cannabinoid inhibition of adenylyl cyclase in regulating voltage-dependent potassium channels.

Using potent ligands such as CP-55,940 and WIN 55,212-2, which are 10-100 times more potent than THC in binding to cannabinoid receptors, Pacheco *et al.,* 1991 showed that cannabinoid agonists inhibited adenylyl cyclase in brain membranes to a significant extent. The cerebellum contained the highest level of cannabinoid-inhibited adenylyl cyclase and also displayed a high level of cannabinoid-stimulated GTPase, which is a direct measure of receptor-coupled G-protein function. Cannabinoid agonists stimulated GTPase in cerebellar membranes by 80-100%, which is the highest level of receptor-stimulated GTPase. activity reported in brain membranes. Such a high level of receptor-coupled G-protein function reflects the relatively high levels of cannabinoid receptors present in brain.

Since cannabinoid-inhibited adenylyl cyclase was absent in mutant mice where cerebellar granule cells were specifically absent (Pacheco *et al.,* 1993), Childers hypothesized that these receptors existed primarily on granule cells in the cerebellum. In cultured granule cells, cannabinoid agonists inhibited cAMP levels with the same pharmacological specificity as in receptor binding studies, with analogs like CP-55,940 and WIN 55212-2 being most potent, and THC being only moderately potent These agonists also inhibited glutamate release from granule cells, thus showing that cannabinoid receptors are probably pre-synpatic in these cells. Other $G_{i/o}$ linked receptors, including $GABA_B$ receptors, also exist in cerebellar granule cells. When cannabinoid and $GABA_B$ receptor function was assayed simultaneously in these cells, agonists for both receptors demonstrated non-additivity in inhibition of cAMP levels. However, cannabinoid and $GABA_B$ agonists were additive in stimulating GTPase in cerebellar membranes. These results demonstrate the phenomenon of receptor convergence. In this case, $GABA_B$ and cannabinoid appear to share common adenylyl cyclase catalytic units although they do not share common G-proteins. Such convergence shows how agonists acting at different receptors can provide similar effects in certain types of cell systems.

One important unanswered question involves the role of cannabinoid inhibition of adenylyl cyclase in regulating neuronal function. Although cannabinoid receptors are coupled to calcium channels, their mechanism apparently involves direct coupling of the receptor to calcium channels via G-proteins, without any diffusible second messenger system. Recent data from Dr. Deadwyler's laboratory have shown that cannabinoid receptors are coupled to another type of ion channel: the voltage-dependent potassium A channel. In cultured hippocampal cells, cannabinoid agonist increased potassium A current (Deadwyler et al., in press) by shifting the voltage-dependent inactivation and activation curves. This effect was mediated through G-proteins, since it was blocked by pertussis toxin and mimicked by GTP-γ-S. The actions of cannabinoid agonists on this current were blocked by addition of 8-Br-cAMP, but were still evident in the presence of forskolin. These results are consistent with the ides that cannabinoid effects on potassium A current are mediated by inhibition of adenylyl cyclase. In this concept, cAMP stimulates protein kinase A to increase phosphorylation of the potassium A channel. The phosphorylated channel would be inactivated, so that decrease in phosphorylation by cannabinoid-inhibited adenylyl cyclase would increase potassium conductance through this channel. These results confirm the importance of this second messenger system in mediating effects of cannabinoid receptors in neurons.

Because cannabinoid receptors are members of the G-protein-coupled superfamily of receptors, it is probable that endogenous ligands exist in brain for these receptors. One such ligand, anandamide (an ethanolamine amide of arachidonic acid), has already been reported (Devane *et al.,* 1992). Childer's laboratory has also been active in isolating endogenous cannabinoids from brain extracts. One compound has been isolated from acid extracts of bovine brain. This compound is relatively polar in nature, is relatively small in size (MW between 100 and 500) and is apparently non-peptide in nature. Although its structure is not yet known, its chemical and chromatographic behavior clearly demonstrates that this compound is different from anandamide. In addition, this compound is relatively specific for cannabinoid receptors, since it displaces the binding of two chemically unrelated cannabinoid radioligands, [^{3}H]WIN-55212-2 and [^{3}H]CP-55,940, to rat cerebellar membranes, and it has no effect on the binding of radioligands to four other G-protein-linked receptors. Future studies will demonstrate whether this compound is structurally related to anandamide or other putative endogenous cannabinoids.

CANNABINOID RECEPTOR BINDING ACTIVITY OF NEUROMODULATOR-LIKE LIGANDS

The identification and characterization of the cannabinoid receptor has recently been accomplished, and the conserved localization within the CNS, its presence on neurons, and its coupling to G-proteins are properties typical of a receptor for neuromodulators (Howlett *et al.,* 1990). Cogent arguments for a natural agonist for the cannabinoid receptor in the CNS have been posited ((Howlett *et al.,* 1992)). Among these include the argument that all G protein-coupled receptors studied to date require association with a ligand for activation. Although ligands which bind to a large number of neuromodulator receptors have been screened for their ability to bind to the cannabinoid receptor, no obvious neuromodulator competes effectively for binding to the cannabinoid receptor at concentrations at which it would be expected to bind to its identified receptor (Bidaut-Russell *et al.,* 1990; Howlett *et al.,* 1992; Kuster, 1993).

Following the premise that an endogenous cannabinoid receptor ligand should have the properties of a neuromodulator being stored in intracellular vesicles, the ability of increased intracellular Ca^{2+} to stimulate release from rat brain slice (250 X 250 μm) was examined in Howlett's laboratory. The displacement of [^{3}H]CP-55940 binding to cannabinoid receptors in rat synaptosomal membranes was the primary assay. The $Ca2^{+}$ ionophore, A23187, released

cannabinoid receptor binding activity in the presence but not in the absence of Ca^{2+} in the media (Evans *et al.,* 1992). The effect of A23187 was maximal at 1.2 µM, consistent with vesicular release. It was necessary to increase. the concentration of extracellular free Ca^{2+} to > 60 nM in order to evoke release. The released cannabinoid receptor binding activity displaced [^{3}H]CP-55940 in a concentration-dependent manner.

The endogenous cannabinoid receptor binding activity also could be released in response to a depolarizing stimulus (75 mM K^+) in the presence of extracellular Ca^{2+} (Evans, *et al.,* submitted). K^+-evoked release was not observed in the absence of extracellular Ca^{2+} and was reduced by over 50% in the presence of either of the specific L and N calcium channel blockers, verapamil and **ω-**-conotoxin, respectively. The efflux of cannabinoid receptor binding activity is greatest within 5 to 10 min of stimulation and receptor binding activity was enhanced by the presence of cocktail of peptidase inhibitors. However, these agents were not effective if a 10-min release period were examined. Examination of the contribution of individual inhibitors revealed a specificity for captopril and thiorphan, inhibitors that act on angiotensin converting enzyme and enkephalinase. These data are consistent with vesicle-mediated release from neuronal terminals. It can be speculated that the factor(s) responsible for competing for [^{3}H]CP-55940 binding may include peptide that are vulnerable to common enzymes responsible for the degradation of a number of neuropeptides in the CNS and other tissues. The specificity of the released factor(s) for the cannabinoid receptor was corroborated by the ability to compete with the aminoalkylindole radioligand [^{3}H]WIN-55212 for binding to this receptor.

The properties of the released cannabinoid receptor binding activity are consistent with its being a relatively small neuropeptide. The released material retains cannabinoid receptor binding activity after boiling and treatment at acid pH. It is able to pass through filters having nominal molecular weight cut-offs of 1000 Da (Evans *et al.,* 1992). The possibility that the responsible factor(s) may be a small peptide is supported by the augmentation of ligand binding activity by inclusion of peptidase inhibitors in the brain slice release preparation (Evans *et al.,* submitted). Thus, Howlett proposed that the cannabinoid receptor agonist in the brain is a small peptide that is stored in vesicles within nerve terminals such that release can be. evoked by depolarization in a Ca^{2+}- dependent manner.

IDENTIFICATION OF ENDOGENOUS CANNABINOID LIGANDS

The approach taken by Mechoulam's laboratory towards the isolation of the endogenous cannabinoid in the brain was based on the assumption that it is a lipid soluble compound. The SAR's in the cannabinoid series indicate that increases in liposolubility, at least up to a certain point, lead to increases in cannabimimetic activity. On this somewhat tenuous basis they assumed that the endogenous cannabinoid ligands were lipids. However, in view of the identification of peptides as modulators of a long list of receptors (including the opiate receptor) the decision to put most of their effort on the lipid soluble fractions was essentially intuitive.

Porcine brains were extracted with solvents appropriate for lipid constituents, namely chloroform-methanol. Several separation routes were investigated. One of them was direct silica gel chromatography of the extract. Numerous problems were encountered. The lipid content of the brain is very high; the extracts presumably contained only minuscule amounts of active material in a complex mixture of various types of lipids which on chromatography possibly served as co-elutants and the active compound therefore was dispersed throughout the chromatographic fractions. By repeated chromatography on silica gel (normal phase and later reverse phase), Mechoulam and his colleagues were able gradually to concentrate the active fractions. A problem which became obvious only towards the end of the endless purifications was the lability of the product: Seldom were they able to elute from the column the amount of active material deposited on it.

The activity was monitored by the ability of fractions to displace a radiolabeled probe, [^{3}H]HU-243, in a centrifugation-based ligand binding assay. However, HU-243, like many cannabinoids. binds to the siliconized polypropylene microfuge tubes in which the assay was conducted. This 3-way equilibrium of the probe among the synaptosomal receptors, the solution and the microfuge tube was a major obstacle in the interpretation of the binding values. Ultimately a fraction was isolated which gave one spot on TLC and eluted mainly as one peak on gas chromatography. This constituent inhibited the specific binding of [^{3}H]HU-243 in a manner typical of competitive ligands with a Ki value of 52±1.8 nM. As mentioned above Pertwee and his collaborators have found that THC inhibits the twitch response of isolated murine vas deferens. Anandamide also elicited a concentration-dependent inhibition of the twitch response.

The laborious isolation procedures described above led to several hundred micrograms of purified material. The isolation of very small amounts of a natural constituent from a complicated mixture inevitably poses problems for the structural elucidation, due to the presence of minor impurities associated with the isolation process. Anandamide, as ultimately isolated, contained traces of materials that originated from the plastic labware as well as traces of solvents, tightly bound to the viscous anandamide.

The structure of anandamide was deduced from mass spectrometric (MS) and nuclear magnetic resonance (NMR) measurements. The initial measurements were by high-resolution MS which suggested the elemental composition $C_{22}H_{37}NO_2$ (m/z 347.2762). that indicates the presence of five double bonds (or rings). The first indication of the structure was the observation that NMR peaks. presumably of double bond protons. were coupled with peaks which were assumed to be signals of doubly allylic protons. Such protons, their couplings and the ratio of vinylic to doubly allylic protons, are typically observed in all-cis, non-conjugated polyunsaturated fatty acids.. At this point Mechoulam and his group assumed that they had a N-derivative of such a fatty acid. Further NMR and MS spectra led to the assumption that anandamide is the ethanolamide of a C-20 fatty acid with four unconjugatcd double bonds, presumably arachidonic acid. This assumption was proved by a simple synthesis.

Shortly after the initial publication on anandamide they published the first report on its in vivo pharmacological activity. The cannabinoids produce a unique syndrome of behavioral effects in animals: at low doses a mixture of depressant and stimulatory effects is observed, while at higher doses central depression predominates. Anandamide exhibited the same profile. At a low dose it caused significant stimulation in the open field. At higher doses, anandamide produced significant depression in the open field, catalepsy, hypothermia and analgesia. In collaboration with Z. Vogel and Y. Barg of the Weizmann Institute they have shown that like THC, anandamide inhibits forskolin-stimulated adenylyl cyclase in $N_{18}TG_2$ cells. The same effect was observed with transfected cells but not with non-transfected cells.

Recently they were able to isolate two additional active ethanolamides (EA's) of fatty acids: the EA's of homo**γ**-linolenic acid and of 7,10,13,16-docosatetraenic acid. Their level of binding to the receptor is very close to that of anandamide. The presence of several fatty acid ethanolamides indicates that the brain produces a familv of endogenous cannabinoid ligands rather than a single mediator of activity. This parallels the situation with prostaglandins, leukotrienes and enkephalins. The exact biological profile may yet reveal subtle differences in the activity of individual members of this new biochemical family.

PHARMACOLOGICAL PROFILE OF ANANDAMIDE

The preliminary reports on the pharmacological properties of anandamide indicate that it produces effects similar to those of Δ^9-THC (Fride and Mechoulam, 1993). Mattin and his colleagues have also examined anandamide's effects in mice. Anandamide was administered

i.v. to male ICR mice in order to evaluate its effects on spontaneous activity (5-15 min), tail-flick response (20), rectal temperature (60) and immobility (90) at the times indicated in parenthesis. Anandamide produced a dose responsive inhibition of spontaneous activity with an ED_{50} of 24.9 mg/kg. Anandamide also produced antinociception in a dose related manner with an ED_{50} of 13.9 mg/kg. On the other hand, it produced less than 10% immobility and less than a 1 °C decrease in rectal temperature at doses up to 60 mg/kg. A few deaths were obtained at the 60 mg/kg intravenous dose. Gross observation of the animals suggested that anandamide had an onset and duration of action which was more rapid than that of Δ^9-THC. A time course study revealed that maximal antinociception and hypoactivity occurred within 15 min. of an i.v. injection of 60 mg/kg. The greatest immobility (40%) was measured at 5 mm and dissipated within 1 hr. The pattern of hypothermia was somewhat different in that the greatest decrease (=2 °C) was found at 15 min which gradually returned to baseline levels between 1 and 2 hr. Reexamination of the e results indicate that anandamide is capable of producing effects similar to those of Δ^9-THC in mice despite being at least 15 times less potent than Δ^9-THC and having a shorter duration of action.

Dr. Welch has also evaluated anandamide for antinociceptive effects following intrathecal (i.t.) administration. The peak effect in the tail-flick procedure (ED_{50} =77 µg/mouse) occurred 3 min following injection. The ED_{50} for Δ^9-THC (i.t.) is 45 µg/mouse. Anandamide was approximately 10 times less potent than Δ^9-THC in the PPQ test. The ED_{50}'s were 3 and 30 µg/mouse for Δ^9-THC and anandamide, respectively. No blockade of anandamide (200 µg/mouse) was observed following pretreatment with naloxone (20 µg/mouse, i.t. or 10 mg/kg, s.c.), nor-BNI (70 ug/mouse, i.t.), ICI 174,864 (20 µg/mouse, i.t.), 8-chloro-c-AMP (10 ug/mouse, i.t.), forskolin (10 ug/mouse, i.t.), or apamin (10 ng/mouse, i.t.). The antinociceptive effects of anandamide were totally blocked by pretreatment of the mice for 7 days with 0.5 µg/mouse pertussis toxin administered i.t. Anandamide produces antinociception following i.t. administration which differ in potency and profile of action from the antinociceptive effects produced by Δ^9-THC.

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INNOVATIVE APPROACHES TO DRUG ABUSE TREATMENT

J. V. Brady

The presentations by the six participants in this symposium focused on a range of research and demonstration initiatives to enhance treatment in patients who, for the most part, were involved with multiple drugs of abuse including both heroin and cocaine. Karst Besteman opened the proceedings with a report on a study to define the differential impact of two levels of treatment services on a randomly assigned patient population which mirrors the demographics of those individuals treated in the local publicly funded drug abuse treatment system of Washington, D.C. Two clinics with defined service differences and different levels of resources are involved in the study focusing upon treatment outcome, effectiveness, and cost-benefit/effectiveness. The general design of the studies provides for randomized patient selection by a NIDA grantee research group (KOBA Associates) operating in conjunction with the central intake system of the District of Columbia (Alcohol and Drug Abuse Services Administration). Both process and outcome (in-treatment and post-treatment progress) evaluations are as well provided by an independent NIDA grantee research evaluation group (Research Triangle Institute) responsible for both the design and administration of evaluation instruments, methods, and procedures. Over 400 patients have been enrolled in the two clinics which differ primarily in their counselor to patient ratios and the level of ancillary services provided (vocational guidance, wellness support. psychiatric intervention). A focus on physical status has produced a number of frequently diagnosed conditions with medical referrals approximating a rate of 10% of the active patients each month. The expected rates of HIV, TB, and other chronic conditions are consistent with the population the two clinics serve. Methadone maintenance with intensive counseling intervention characterizes the major treatment modality in both clinics with comparisons based upon patient attrition through drop out and non-compliance. Early findings confirm that drug use among patients in both clinics has decreased dramatically with 70% and 50% of the patients in each clinic respectively, providing drug-free urine test analyses.

Extending the theme of clinical issues in drug abuse treatment, David Novick addressed the topic of long-term methadone maintenance citing the lack of published studies on patients with five or more years of treatment. The presentation focused on three important issues regarding the clinical status of such patients: 1) physiological normalization, 2) medical safety, and 3) treatment modification. In the first instance, a comprehensive medical evaluation study was performed on a randomly selected sample of methadone patients admitted to treatment between 1965 and 1968 who remained in treatment for 11 to 18 years (median 14.4 years). Based upon previous findings by Kreek and colleagues suggesting that neuroendocrine (ACTH. cortisol, and beta-endorphin) and immunologic (natural killer activity, lymphocyte subsets, and immunoglobulin levels) parameters become normalized during long-term methadone maintenance in former heroin abusers, the patients in the study sample were compared to an untreated control group of 56 long-term heroin abusers. The findings confirmed that with regard to medical diagnoses, symptomatic complaints, physical examination results, and laboratory test outcomes there was little difference between the long-term methadone patients and the untreated heroin abusers. Moreover, from the perspective of medical safety, the results showed that successful long-term methadone patients who are employed, have completed the counseling process, and are no longer using illicit drugs or alcohol can be effectively treated in a physician's office rather than in a licensed drug abuse treatment clinic. A follow-up study of such methadone maintenance in the context of general medical practice has shown high retention rates up to nine years with few management problems in a

carefully selected group of patients. These studies have also provided the basis for developing new treatment modification programs for other groups of long-term methadone maintenance patients based upon the application of laboratory, clinical, and evaluation research findings that confirm the safe and effective use of such extended pharmacological interventions.

Despite the demonstrated success of long-term methadone maintenance treatment in highly selected groups of heroin abusers, reported one-year retention rates of patients admitted to methadone maintenance programs in general vary widely from 34% to 89%. The variations in retention can be presumed to arise as a result of variations in patient, program, and other situational factors with several reports suggesting that required treatment fees impair retention on methadone maintenance. James Maddux described the results of a prospective study designed to evaluate the effects of such treatment fees on retention in a methadone maintenance treatment program. One hundred and fifty-two (152) illicit opioid abusers were admitted to the treatment program and randomly assigned to a fee or no-fee condition, even though virtually all the patients expressed a desire to enter the study because of the possibility of free treatment. The patients in the fee group were required to pay $2.50 per day during the course of treatment while the patients in the-no-fee group paid nothing. All patients were followed for one year with interviews obtained from all but two of the patients who had died. The results showed clearly that the no-fee group had significantly greater retention rates than the fee group. The one year retention rate for the patients required to pay the $2.50 daily fee was only 34% compared to 54% for the patients in the no-fee group. There were however, no significant differences between the degree of improvement in those patients who remained in treatment for the one year period regardless of whether they were in the fee or no-fee group. As measured by urine test results and subject-reported intravenous drug use, crime and incarceration, only small, non-statistically-significant differences between the two groups were found. The patients in the no-fee group did however, report significantly more days of productive activity than the patients in the fee-paying group. The age of the patients was the only one of 21 personal/demographic variables that was significantly related in a positive direction to retention rate. A measure of the maximum methadone dose level during treatment was also positively correlated with retention rate while the number of interviews per month with a caseworker was found to be inversely related to retention rate. It seemed likely however, that both the increased number of caseworker interviews and the decreased retention rate may have been a function of the severity of a third factor, the problems for which the patients sought treatment.

Among the many general health status problems that substance abuse treatment programs are increasingly called upon to address, the need to provide services for patients who are infected with the human immunodeficiency virus (HIV) is perhaps the most serious. Steven Batki described an innovative substance abuse treatment approach integrating medical and psychiatric service for such HIV-infected drug abusing patients. The common finding of psychiatric co-morbidity was confirmed in a series of 84 HIV-infected intravenous drug abusers in methadone maintenance treatment for more than six weeks at the San Francisco General Hospital Substance Abuse Services, 65% of whom required psychiatric consultation. Those requiring psychiatric consultation were found to have higher rates of continued drug abuse and lower rates of zidovudine (AZT) use, though daily on-site dispensing did significantly improve AZT adherence. A prospective study of 75 HIV-infected drug abusing patients at the San Francisco General Hospital did show that methadone maintenance treatment was associated with significant reductions in drug use even in this compromised patient population. Poor outcomes after 12 months in methadone maintenance treatment were however predicted by cocaine use at intake and confirmation was provided that cocaine abuse is more treatment-resistant than opiate abuse in HIV-infected methadone maintenance patients. On the other hand, a controlled trial of fluoxetine for cocaine dependence in HIV-infected intravenous drug abusers at the San Francisco General Hospital was able to demonstrate that such an intervention was both safe and at least to some extent, effective in reducing cocaine use as well as depression in this patient population. The results of these several studies confirm that treatment for HIV-

infected intravenous drug abusers should integrate psychiatric, HIV-medical, and substance abuse related services and that additional research can be expected to yield safer and more effective substance abuse treatments for such patients.

Among the most important factors that determine the effectiveness of drug abuse treatment programs in general and methadone maintenance treatment in particular are the accessibility of essential services and the length of time that patients are maintained in contact with those services. Joseph Brady described a demonstration research project that has as its objective the development, maintenance, and evaluation of an innovative mobile heath service delivery system approach to the enhancement of drug abuse treatment. The focus of the project has been upon the identification and recruitment of intravenous drug abusers into a treatment program that provides health education, outreach, and clinical support to targeted inner city communities in Baltimore, Maryland with a high prevalence of substance abuse and other poor health status indicators. Two mobile units, each consisting of a medication dispensing van and a house trailer modified to serve as a counseling and general clinical services unit, provide the treatment. One unit visits multiple locations in west Baltimore each day and the second unit remains stationary for the entire day in a demographically comparable area in east Baltimore. Over 500 intravenous opioid abusers have applied for admission to the program during its first year of operation and some 200 patients have been actively enrolled in treatment with a gratifying low drop-out rate approximating less than 15%. Both process and outcome evaluations completed to date confirm the savings in time and money reflected in the patient self-reported comparisons between their previous drug abuse treatment programs and the mobile health service drug abuse treatment program. Urine sample measures have also shown a marked decrease in drug use during treatment compared to pretreatment and intake drug use levels. Self-reported levels of legitimate employment have increased from less. than 20% at intake to over 35% during the first 6-month course of treatment and comparisons with other fixed site drug abuse treatment programs in the city of Baltimore suggest a range of differential process and outcome effects. The results of this comparative analysis show that the mobile health service had a higher percentage of patients reporting daily drug use on admission; fewer previous drug abuse treatment admissions; and an average length of stay in the mobile health service treatment program was greater than for fixed-site programs.

In a discussion of these several presentations, John Ball called particular attention to the innovative aspects of both the methodological and substantive contributions described and emphasized the need for continuing research evaluations to expand the data base upon which the safety and efficacy of improved drug abuse treatment must depend.

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THE ROLE OF EXCITATORY AMINO ACIDS IN THE ACTIONS OF ABUSED DRUGS

J. F. McGinty

The participants in this symposium discussed the interactions between dopamine and excitatory amino acids (EAA) in the CNS pathways affected by abused drugs. Many of the actions of alcohol, opiates, and psychostimulants are mediated by EAA receptor stimulation. The focus of this svmposium. however. was entirelv on nsvchostimulants because of the explosion of research during the last four years which has demonstrated that N-methyl-D-aspartic acid (NMDA) and non-NMDA EAA receptor antagonists block the motor stimulating, neurotoxic, and rewarding actions of cocaine and the amphetamines.

The subjects covered by the speakers included (1) identification of the CNS sites in which locally infused EAA receptor antagonists exert anti-stimulant actions, (2) the ability of dopamine agonists to release glutamate, (3) the ability of competitive and non-competitve NMDA antagonists to block the dopaminergic neurotoxic actions of methamphetamine, (4) blockade of cocaine- and methamphetamine-induced increases in striatal neurotensin and dynorphin expression, and (5) a theoretical framework in which to interpret dopamine-glutamate interactions based on proposed tonic and phasic dopamine release in the striatum.

The functional organization of the neurochemical/anatomica1 circuitry important in stimulant-induced reward and motor behaviors was reviewed by McGinty and Kalivas. The dorsal striatum receives dopaminergic input from the substantia nigra pars compacta and glutamaternic input from widespread areas of the cerebral cortex. Its GABAergic outputs are to-the globus pallidus'and substantia nigra with striatopallidal outputs containing enkephalin and striatonigral outputs containing dynorphin and tachykinins. The dopamine/EAA innervation of the ventral striatum is organized in a similar manner. Besides the dense dopaminergic projection to the nucleus accumbens (NAc) from the ventral tegmental area (VTA), glutamatergic input arises from three major sources: the prefrontal cortex, amygdala, and hippocampal formation. Output from the nucleus accumbens includes GABAergic projections to the ventral pallidum and VTA. These projections also contain neuropeptides, including the opioid peptides, enkephalin and dynorphin, as well as tachykinins. The VTA also sends dopaminergic input to the prefrontal cortex which, in return, sends a glutamatergic projection back to the VTA. Therefore, there are two major CNS sites in which dopamine/EAA interactions may mediate, the response to psychostimulants: the NAc and the VTA.

Psychostimulant-induced dopamine uptake blockade and increased release into the synapses of the dorsal and ventral striatum stimulate dopamine receptors of all types. However, most is known about the consequences of Dl and D2 receptor stimulation. Dl receptor stimulation leads to increased immediate early and dynorphan gene expression in the striatonigral pathway whereas increased D2 receptor activity has little, if any, effect on enkephalin gene expression in the striatopallidal pathway. These changes in gene expression are attenuated by NMDA receptor antagonists. Although the involvement of EAAs in the acute effects of stimulants is controversial, strong evidence exists that behavioral sensitization to repeated administration of stimulants is mediated by glutamatergic systems. Preliminary evidence indicates that increased glutamate release as well as upregulation of EAA receptors may be involved.

Karler (1989) initially reported that amphetamine and cocaine-induced behavioral

sensitization could be blocked by prior administration of the non-competitive NMDA receptor antagonist, MK-801 (dizocilpine maleate). Further research by Karler's group (Karler 1991) and others has begun to discriminate between the role of NMDA and non-NMDA receptors in the induction and expression of stimulant-induced sensitization. To date, the effects of psychostimulants which have been inhibited by EAA receptor antagonists include (1) behavioral sensitization (locomotor activity and stereotypies), (2) convulsions, (3) dopaminergic neurotoxicity, (4) neuropeptide alterations, and, most recently, (5) rewarding properties of cocaine and amphetamine.

Peter Kalivas reviewed the current thought on the circuitry and neurochemical mechanisms involved in behavioral sensitization (Kalivas 1991; 1993). He presented recent data that MK801 infused into the VTA or amygdala, but not into NAc, blocks cocaine-induced sensitization (Alesdorf and Kalivas in press). In addition, he discussed evidence indicating that repeated cocaine exposure results in a blunting of the response of mesocortical DA to a cocaine challenge dose seven days later. These data may indicate that the inhibitory tone of mesocortical dopamine on glutamate release is diminished with resultant disinhibition of the prefrontal cortical EAA influence on VTA. Finally, Kalivas' preliminary data indicates that administration of a full Dl agonist into VTA causes an increase in extracellular glutamate as detected by microdialysis. Thus, a portion of Dl receptors may reside on prefrontal cortical glutamatergic endings in the VTA in addition to their presence on NAc-VTA terminals.

Pat Sonsalla discussed the role of NMDA receptors in dopaminergic neuronal degeneration in methamphetamine-treated mice. Sonsalla and colleagues (1989; 1991) have demonstrated that competitive and non-competitive NMDA receptor antagonists block methamphetamine-induced neurotoxicity. However, Sonsalla's group did not observe any effect of MK801 on methamphetamine-induced dopamine release in contrast to the data of Weihmuller and colleagues (1991). In addition, Sonsalla discussed possible reasons why dopamine terminals in the caudate are selectively vulnerable to methamphetamine-induced degeneration. Finally, Sonsalla contrasted the role of NMDA receptors in methamphetamine-induced toxicity with that in MPTP- and MPP^+-induced toxicity. Although Turski and colleagues (1991) had reported successful blockade of MPP^+ effects by MK-801, Sonsalla's data indicate that this form of dopaminergic toxicity is less sensitive to NMDA receptor blockade (Sonsalla and Nicklas 1992).

Glen Hanson reviewed data generated in his lab that methamphetamine and cocaine cause D1- mediated increases in dorsal and ventral striatal neurotensin and dynorphin which are blocked by MK801 (Johnson *et al.,* 1990; Singh *et al.,* 1991). He focused his attention on stimulant-induced neurotensin changes, which are particularly robust in the medial caudate and NAc and the fundus striata, because there is evidence that neurotensin administration is functionally antagonistic to the actions of psychostimulants (Nemeroff *et al.,* 1985). Conversely, subconvulsive stimulation of NMDA receptors or blockade of D2 receptors increases neurotensin-immunoreactivity in the striatum. His data indicate that NMDA receptors are necessary for D1, but not D2, receptor stimulation of these peptide systems in limbic and motor striatum and substantia nigra, changes that may contribute to the actions of psychostimulants.

Tony Grace provided evidence for EAA/dopamine interactions in the NAc and introduced a theoretical framework (Grace 1991) in which to interpret such interactions in response to psychostimulants. He introduced evidence that D2 receptor agonists attenuate EPSP's in the NAc evoked by stimulation of glutamatergic inputs *in vitro* and that multiple stimulation of these afferents; which augments glutamate release, causes an increase in D2-mediated attenuation of these EPSPs. Furthermore, Grace proposed that tonic dopamine release, which is locally regulated by D2 and NMDA receptor activity at the presynaptic dopamine terminal, and phasic, or action potential-dependent, dopamine release are affected differentially by acute, chronic, and withdrawal phases of psychostimulant administration.

The full proceedings of this symposium will be published together in an upcoming issue of *Drug and Alcohol Dependence.*

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TOLERANCE AND SENSITIZATION TO OPIOIDS AND COCAINE

J. U. Adams, S. Izenwasser, T. H. Kramer, C. W. Stevens, P. J. Tiseo and E. M. Unterwald

An important and fascinating feature of addictive processes is the alteration in drug sensitivity which often occurs with repetitive use of the drug. Opioids and cocaine are especially notable in this regard. The effects of chronic administration of opioids or cocaine are manifested in biochemical changes, alteration in the shape and position of drug dose-response curves in animals, and changes in the magnitude of effects of drugs administered to humans for purposes therapeutic or otherwise. In this Young Investigator's Symposium, researchers integrate findings from a variety of perspectives on the pharmacology of opioids and cocaine.

TOLERANCE TO OPIOIDS

The Relationship Between Opioid Tolerance and Dependence

Jill U. Adams

The dissociation of tolerance and dependence is often reported. Many factors can influence the degree of tolerance and dependence observed and may account for apparent separation of the two processes. The purpose of this presentation is to review some of these factors.

First, the experimental methods used to measure tolerance arc quite different than those typically used to measure dependence. For example, tolerance to morphine-induced analgesia is often compared to a naloxone-induced syndrome of gross behavioral signs indicative of withdrawal. Even when a single. baseline is used to measure both effects, there are fundamental differences in the actual changes being measured. For instance, tolerance is a decreased sensitivity to an agonist-induced effect whereas dependence is the presence of an antagonist-induced effect where before there was none. Moreover, tolerance requires the presence of the agonist to be measured, whereas dependence requires its absence (the principle of uncertainty, Collier, 1984). These fundamental differences may make definitive association or dissociation of tolerance and dependence difficult to experimentally demonstrate Second, depending on the sensitivity of the assays, the magnitude of tolerance and dependence may differ and this may account for one effect persisting in the apparent absence of the other.

Third, the contribution of non-pharmacodynamic factors, namely behavioral conditioning, may alter the expression of tolerance and dependence. The role of behavioral conditioning in opioid tolerance has been elegantly characterized by Siegel (1983). It can be argued that even differential interactions with behavioral influences might indicate differences in mechanism. For example, different signs of withdrawal can be differentially modified by environmental stimuli. Monkeys have been conditioned to emit some but not all signs normally associated with opioid withdrawal upon presentation of a cue previously paired with nalorphine injections (Goldberg & Schuster, 1970). Regardless of behavioral factors, tolerance to different effects of opioids develop at different rates. There is the classic example of analgesic tolerance occurring in the absence of tolerance to the inhibition of gastrointestinal transit. Thus, it would appear that tolerance (or dependence) is dissociable from itself. In light of this, how relevant arc conclusions of dissociation of tolerance from dependence?

In conclusion, while opioid tolerance and physical dependence may be dissociable several factors may be taken into account to evaluate the relevance of such a result. The great majority of data regarding the relationship between tolerance and dependence suggest that, in general, the two processes are closely related.

TOLERANCE TO OPIOIDS IN AN AMPHIBIAN MODEL

C. W. Stevens

Opioid administration produces a profound and long-lasting analgesia in humans and in a variety of pain models using other mammalian species. However, opioid analgesia is not limited to mammals as analgesic or antinociceptive effects have been observed after morphine administration in lower vertebrate and invertebrate animal models (Kavaliers 1988, Steve 1992). With regard to the amphibian model, we have developed a behavioral assay for opioid action based on the application of diluted acetic acid to the frog hindlimb, called the acetic acid test. Results using the acetic acid test demonstrate that amphibians exhibit long-lasting and dose-dependent analgesia following systemic administration of morphine (Pezalla 1983; Pezalla and Stevens 1984; Benyhe and Wollemann 1988; Benyhe *et al,* 1989; Stevens and Pezalla 1989), levorphanol (Pezalla and Stevens 1984). and oxymorphazone (Benyhe *et al.,* 1989). Additionally, direct administration of opioids to the frog spinal cord by intraspinal injection produces a potent and dose-dependent analgesia as shown following administration of morphine and other alkaloids (Stevens and Pezalla 1983, Stevens and Pezalla 1984. Stevens and Pezalla 1989. Stevens 1991a). met-enkephalin. beta-endorphin, and dynorphin (Stevens *et al.,* 1987) and highly selective opioid agonists (Stevens 1991b). Finally, amphibians appear to possess a well developed endogenous opioid system, replete with high concentrations of opioid binding sites and endogenous opioid peptides (Stevens 1988). While the above suggest that the mechanisms of opioid action in amphibians may be similar to that observe humans and other mammalian species, there has not been a systematic examination of opioid tolerance in the amphibian model.

Daily bolus injection of about an ED85 dose of morphine (100 nmol/g.s.c.) or saline (10 µl/g) for one week resulted in a significant analgesia for three days in the morphine group, which fell to levels indistinguishable from the saline-treated controls on days 4 through 7. In separate experiments, animals were treated identically with morphine or saline but were not tested daily for pain thresholds. In these animals, administration of a range of morphine doses on day 8 yielded dose-response curves significantly shifted rightward by a factor of 3.3 in the morphine-treated group compared to the saline-injected controls. These studies are the first to show the time course of tolerance development and the magnitude of morphine tolerance in a non-mammalian vertebrate species. Finally, the ability of amphibians to withstand a wide range of ambient temperature, may allow for unique studies for the rate of opioid tolerance under unique experimental conditions.

CLINICAL TOLERANCE TO OPIOIDS

Paul J. Tiseo

The experimental literature on tolerance, consisting primarily of animal studies and studies with human addicts, is replete with reports demonstrating the progressive loss of effect in the face of steady dosing which results in the need for continual dose escalation to maintain a desired effect. The expected result of such a process in a patient with chronic pain would be a continuous escalation of opioid dosing and an inability to maintain an adequate analgesic effect. Fortunately, long term evaluation of the opioid requirements of cancer patients demonstrates that this process rarely occurs in the clinical setting.

The limited data from clinicaI studies suggest that the phenomena produced in experimental models can occur to some degree in patients. It is evident, however, that the complexity of human pain and its effects on the CNS demands caution in extrapolating the findings from these studies to the clinical situation. In order to address the extent to which patients chronically receiving opioids increase their opioid requirements over time, a number of survey studies have been undertaken to assess the patterns of drug use in patients with cancer pain (for review, see Foley 1993). From these studies, three patterns of drug use emerge which are in contrast to the phenomenology of tolerance as described in the animal and human addict literature and suggest the presence of factors other than tolerance playing a role in this setting. The three patterns are: 1) stable dosing which extends for prolonged periods of time (weeks to months) during which analgesia is maintained in the absence of dose escalation; 2) rapidly escalating opioid doses, where the need for dose escalation can be attributed not simply to tolerance, but to the progression of disease and increasing pain; and 3) the marked reduction of opioid dosing following effective relief of pain by anti-cancer therapies or anesthetic or neurosurgical procedures. Of critical importance in this latter group however, is the observation that failure to reduce the opioid dose following the attenuation of pain will result in the onset of a spectrum of symptoms normally associated with opioid toxicity *(i.e.,* sedation, myoclonus, respiratory depression), demonstrating that in the sudden absence of pain these patients no longer exhibit tolerance to the adverse. effects of their opioid.

It is speculated here that although these patients can tolerate high doses of opioids, they are not tolerant to all opioid effects in the same pharmacological sense that experimental models are following chronic exposure to the drug. It is hypothesized then that it is not tolerance alone, but the "driving force" of pain itself *(i.e.,* nociceptive transmission through the brainstem; increased sympathetic drive) which allows these patients to have near normal autonomic function *(i.e.,* respiration, alertness) in the presence of high doses of opioids. In addition, we would postulate that the presence of pain either prevents or rapidly reverses the cellular changes (*i.e.,* receptor-effector uncoupling) which are suggested to take place in experimental studies in which chronic pain is not present and in which there is a need for continued dose escalation to achieve an effect. One could speculate that this is simply a physiological adaptation which allows cells to remain functional in the face of a persistent nociceptive stimulus. As such, the patient continues to be opioid sensitive, and this is clearly demonstrated by the onset of opioid toxicity in those patients whose pain is abruptly removed and whose opioid dose is not reduced.

SENSITIZATION AND TOLERANCE TO COCAINE

Sari Izenwaser and Ellen M. Unterwald

Cocaine is a psychomotor stimulant that inhibits the reuptake of dopamine into presynaptic dopaminergic neurons. thus increasing synaptic dopamine concentrations. Acute injection of cocaine produces an increase in locomotor activity which becomes progressively greater upon repeated drug administration. Although there have been many studies aimed at determining the neurochemical bases underlying this sensitization or enhanced behavioral response to repeated cocaine, definitive results have not been obtained. Changes in stores of neurotransmitter do not appear to be important, as tissue levels of dopamine in the striatum and nucleus accumbens are not changed after acute or chronic cocaine administration. The effects of cocaine on dopamine receptor densities have been equivocal with reports of increases, decreases, or no change in D_1 and D_2 receptor number.

Cocaine and several other psychomotor stimulants *in vitro* inhibit reuptake of [^{3}H]dopamine into presynaptic terminals. Repeated daily cocaine injections (15 mg./kg ip once daily for three days) produce behavioral sensitization. result in a persistent decrease in [^{3}H]dopamine uptake *in vitro,* and increase the potency for cocaine inhibition of uptake in rat nucleus accumbens, but not in the caudate putamen (Izenwasser and Cox 1990). *In vivo*

microdialysis studies have shown that an acute injection of cocaine increases extracellular dopamine. This increase is augmented seven days after withdrawal from a repeated dosing regimen (*e.g.,* Akimoto *et al.,* 1989) but attenuated following a shorter withdrawal period of 24-48 hours (Hurd *et al.,* 1989; Segal and Kuczenski 1992).

Under certain conditions, tolerance rather than sensitization develops to behavioral and neurochemical effects of cocaine. Continuous cocaine administration via osmotic minipumps produces tolerance to the locomotor-stimulating effects of cocaine (Reith *et al.,* 1987; unpublished data from Izenwasser and Cox 1992a). In addition, tolerance to the effects of cocaine on operant behavior has been observed. Acute administration of cocaine produces dose-related decreases in response rates in animals on an FR 30 schedule of food reinforcement. Repeated daily cocaine administration produces tolerance to this effect, as evidenced by a shift to the right of the dose-effect (Katz *et al.,* 1993).

Cocaine administration also affects the endogenous opioid system. Dynorphn peptides (Sivam 1989) and prodvnorphin mRNA (Spangler *et al.,* 1993: Hurd *et al.,* 1992) are elevated following repeated cocaine exposure.Chronic "binge" cocaine administration (three injections/day at one-hour intervals for 14 day) produces behavioral sensitization and causes upregulation of mu and kappa, but not delta, opioid receptors primarily in brain regions rich in dopamine terminals (Unterwald *et al,* 1992). Alterations in [^{3}H] naloxone binding have been reported following continuous infusion of cocaine (Hammer 1989). Similar to some of the effects on behavior and dopamine uptake. different treatment regimens produce differential effects on opioid receptor function. Chronic "binge" cocaine results in a loss of delta opioid inhibition of adenylyl cyclase activity in both the caudate putamen and nucleus accumbens,with no change in the regulation of this second messenger by mu opioid ligands (Unterwald *et al.,* 1993). In contrast, chronic infusion of cocaine produces an increase in mu opioid inhibition of adenyl cyclase activity in the nucleus accumbens, but not in caudate putamen, and has no effect on delta opioid receptor function (Izenwasser and Cox 1992b).

Thus, chronic administration of cocaine has profound effects on a number of nuerochemical systems. The biological bases, underlying sensitization or tolerance to cocaine is not yet fully understood. There are, however, a number of factors that influence the development of sensitization and tolerance and include drug dose, dosing regimen, length of treatment, time since the last treatment, and the behavioral measure. A better understanding of the effects of different treatment regimens that lead to sensitization of tolerance to cocaine, and the neurochemical changes associated with these effects, should aid in the identification of factors that may be important in the development of treatments for cocaine addiction.

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A reference list is available on request from T. H. Kramer.

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OVERVIEW: ANTISOCIAL PERSONALITY AND TREATMENT RESPONSE IN SUBSTANCE ABUSERS

A.I. Alterman

There is a long-standing belief that substance abusers with a diagnosis of antisocial personality disorder (APD) do not benefit from treatment. A review of the relevant studies indicates, however, that APDs do benefit from treatment, although to a lesser degree than patients with no additional psychiatric disorders (Alterman and Cacciola 1991). The APD diagnosis is not a uniquely negative prognostic indicator. Patients with diagnoses of major depressive disorder as well as those with an additional substance diagnosis benefit less from treatment. A number of studies have shown that APD is a heterogeneous categorization and that there may be subtypes of APD patients with a differential treatment response. It is therefore encouraging that a number of studies are now being conducted to further delineate and clarify the implications of the APD diagnosis in substance abusers. The following papers describe a number of approaches concerned with issues related to APD and its relation to treatment response.

HISTORY OF ANTISOCIAL BEHAVIOR AND TREATMENT OUTCOME IN WOMEN

L. Cottler, W. M. Compton, III and G. A. Asmus

Childhood conduct disorder has been shown to lead to adult antisocial behavior (AAB) and antisocial behavior may predict treatment outcome among substance users. This paper discusses the subtypes of antisocial behaviors and their effects on HIV risk behavior after one year of follow-up. The data came from two studies of the seroprevalence and serconversion among current and former drug users and partners of current users. Both studies had a 12 month follow-up in the protocol. Of the 1080 persons interviewed, there were 262 women; 21% met criteria for Antisocial Personality disorder (APD); 41% met adult criteria only, but not childhood; 36% met neither threshold; and 3% reported childhood only.

Recency of injection drug use and high risk sexual behaviors (such as 10 or more partners, not always using condoms, having a partner who injected drugs, etc.) was evaluated among women with and without adult antisocial behavior (n=162 vs. n=100). Women with AAB, compared to those without, were more likelv to report IDU 133% vs. 13%). but were more likely to change this behavior over 12 months; equally likely to report high risk sexual behaviors, but not more likely to change this behavior (82% vs. 78% recalcitrant); were more likely to use cocaine (86% vs. 53%); and more likely to receive drug treatment (74% vs. 41%). Older white women with AAB and a history of both depression and drug abuse treatment were more likely to be injection drug users at the 12-month follow-up. High risk sexual behaviors at the 12 months follow-up were predicted by AAB only.

Further analyses are needed to evaluate among women the association between AAB and recalcitrant behaviors. However, it appears that women are more likely to change their drug using behaviors than their high risk sexual behaviors.

POST-TREATMENT OUTCOME IN CONDUCT-DISORDERED BOYS

T. Crowley

Youths with Conduct Disorder comprise a sizable proportion of patients with adolescent drug problems. Descriptions of these patients' drug use have been limited, and there have been no reports of the outcome of drug treatment of these patients. We here describe a cohort of such patients admitted to long-term residential care, including information on follow-up of these patients.

Subjects and Methods. Patients were referred by criminal justice and social service agencies throughout Colorado for long term residential care at Synergy, a program of our service. Patients referred to Synergy share these characteristics: serious substance involvement, considerable antisocial behavior, major family turmoil, frequent failures in previous treatments, frequent placements in group homes or detention, not psychotic, and not considered imminently dangerous despite histories of violence, fire-setting, or past suicide attempts.

Synergy is an unlocked, non-hospital residential treatment program offering 6-12 months duration with extensive aftercare available. It operates as a modified Therapeutic Community relying on intensive confrontational groups, individual and family counseling by state certified drug abuse counselors, vocational and recreational activities, and an on-site school.

Assessments included the Diagnostic Interview Schedule for Children (DISC) on about Day 3, the Composite International Diagnostic Instrument - Substance Abuse Module (CIDI-SAM) on about Day 2, the Comprehensive Addiction Severity Index - Adolescents (CASI-A) on about Day 5, the Carroll Self-Rating Scale for Depression, and a modified Aggression Rating Scale on about Day 25. Follow-up interviews using the CASI, Carroll, and DISC were done at 6 and 12 months with all patients who could be tracked and agreed to be paid follow-up participants.

Results. Eighty-five percent of the youths were either White Anglo or Hispanic, and their mean age was 16.2 years. All were male. All patents had clinical diagnoses of Conduct Disorder. All also had Drug Dependence diagnoses by DSM-III-R criteria. The group was highly aggressive on a standardized aggression scale. The prevalence of dependence exceeded 60% for marijuana, for alcohol. and for tobacco, which were the three drugs of highest prevalence in this group.

At one year follow-up the youths reported a sizable drop in the 6 month prevalence rate of Conduct Disorder symptom behaviors, in number of drugs used, and in the days of use of various drugs. The youths reported fewer drug problems at follow-up. There were significant reductions in self rated depression at follow-up, and there was a marked reduction in the prevalence of Major Depressive Disorder at follow-up. There was a striking and highly significant increase in the proportion of youths who had graduated from high school or obtained GED's at one year follow-up. The number who had recently been in jail or detention had fallen dramatically, whereas the proportion living in group homes or therapeutic situations had risen significantly.

There was an average, marked improvement in the clinical status of youths one year after admission to a treatment program. Without a control group it is not possible to ascribe the improvement to the treatment, but it is important to recognize that improvement can occur in violent, conduct disordered, drug dependent youths.

TREATMENT OUTCOMES ASSOCIATED WITH ANTISOCIAL PERSONALITY AND OTHER PERSONALITY DISORDERS.

R. Brooner

Antisocial Personality Disorder (APD) in opioid abusers has been associated with poor treatment prognosis and increased risk of HIV infection (Woody et al. 1985: Brooner et al.. 1992). These findings have stimulated interest in both the diagnosis of APD and in possible subtypes of the syndrome. For example, APD opioid abusers who met criteria for other personality diagnoses also scored higher on measures of neuroticism and psychiatric distress compared to patients who met criteria for APD only (Rousar et al., in press). The present study examined the relation between personality disorder comorbidity in APD opioid abusers and treatment outcome. A total of 344 opioid abusers categorized diagnostically into one of four groups: 1) no personalitv diagnosis - NONE; 2) APD only - Pure; 3) APD plus other personality diagnoses - mixed: and 4) personality diagnosis other than APD - Other. Diagnoses of personality disorder were made using the Structured Clinical Interview for DSM-III-R. Personality traits were assessed using NEO Personality Inventory (NEO-PI) and psychiatric distress was measured using the SCL-90R.

The majoritv of patients were categorized in the None group (N= 231), followed by 54 Pure APD, 21 Mixed'APD, and 38 Other personality disordered patients. The Mixed APD group had a higher Neuroticism domain score compared to both the Pure APD and the Non-Axis II groups ($p<.01$), and significantly higher scores on 5 of the 6 facets of Neuroticism compared to both the Pure APD and the None groups (e.g., Anxiety, Hostility, Depression, Self-Consciousness, and Vulnerability). The Mixed group also reported significantly higher state levels of distress on each of the SCL-90 subscales compared to the Pure and None groups. No significant differences on these measures were found between the Mixed and the Other group. This data indicate that Axis II comorbidity among APD opioid abusers is associated with higher levels of emotional distress and instability.

The impact of chronic, pervasive dysphoria reported by Mixed APD patients and treatment outcome was examined by comparing their intake and 7-month Addiction Severity Index (ASI) composite scores (CSs), treatment survival rate, and percent of urine specimens positive for illicit drugs to Pure APD patients and those in the remaining two groups. There was a modest trend for greater treatment retention for Mixed APD group compared to patients in the Pure APD group and the Other Groups (63% vs. 52% and 50%. respectively). The Mixed groups also had significantly higher intake AS1 CSs on the medical and alcohol scales compared to the three remaining groups, and a significantlv higher psvchiatric CS than the Pure group (.15 vs. 05). AIthough the Mixed group had lower medical and alcohol CSs at month 7 compared to intake these differences were not statistically significant. In contrast, both the Pure APD and the None groups reported significantly lower drug CSs at month 7 versus intake. Urine results for opiods and cocaine revealed an interesting picture. The Mixed group had the lowest rate of opioids positive urines (10%). with the Pure, Other and None groups having very similar positive rates (28%, 25%, 25%, receptively); this difference was not significantly significant. The Pure APD had a significantly higher percent of cocaine positive urines compared to the None Group (32% vs. 20%. respectively): the Mixed group had the lowest rate (19%) though this difference was not significantly significant. Finally, there was a modest trend for the Mixed group to have a lower overall positive urine rate (i.e., opioids, cocaine, sedatives combined) compared to the Pure APD group (33% vs. 58%).

In summary, a subset of APD patients were found (i.e., Mixed) that were significantly more prone to dysphoria than Pure APD patients, with significantly higher intake ASI medical. alcohol and psychiatric severity scores. The Mixed-APD's had a slightly higher rate of treatment survival and the lower rates of opioid, cocaine and combined urine positives.

THE PSYCHOPATHY CHECKLIST AS A PREDICTOR OF NEGATIVE TREATMENT OUTCOME

A. I. Alterman, J. S. Cacciola and M. J. Rutherford

Introduction. In an ongoing study we are comparing the reliability and predictive validity of the APD diagnosis and the psvchopathy diagnosis derived from administration of the revised Psychopathy Checklist (PCL-R; Hare 1991). There is some indication in the literature that the PCL-R determination may yield a more severe antisocial subtype than APD (Alterman and Cacciola 1991).

Subjects and Method. The subjects were new intakes into methadone maintenance (MM) treatment. All were males. The number of subjects at baseline was 18 1, that at one month was 178, then was 159 at 6 month follow-up and 67 at 24 month follow-up. The PCL-R and an APD interview were administered at 0,1, 6, and 24 months as well as the ASI.

Results. The test-retest reliability (different interviewers at each timepoint) of the APD diagnosis for baseline vs. one month, baseline vs. 6 months and baseline vs. 2 years was 0.39. That for the psychopathy diagnosis (baseline PCL-R score of 25 or more) was 0.72 for the same time points. Thus, the psychopathy diagnosis was more reliable than the APD diagnosis. APD subjects (n=47) had significantly poorer baseline ASI composite scores than non-APD (n=134) subjects in the legal and family/social areas. Psychopathic (PSYCHOP-n=39) subjects had significantly poorer scores in the legal, drug, and employment areas than non-psvchopathic subjects (non-PSYCHOP-n=143). AU groups showed significant improvements in the ASI at six and 24 month follow-ups except for the medical and employment areas. APD and PSYCHOP subjects improved as much as non-APD and non-PSYCHOP subjects. No differences were found for up to seven months of treatment urines for the APD vs. non-APD group. There was a tendency for the urines of PSYCHOP subjects to be dirtier than those of non-PSYCHOP subjects (barbiturates: 11.8% vs. 2.2%; $p<.08$). PSYCHOP subjects also had a higher proportion of dirty cocaine urines than non-' ' PSYCHOP subjects (75.5% vs. 60.2%). but this difference was not statistically different. PSYCHOP subjects were significantly more likely than non-PSYCHOP subjects (47.1% vs. 21.5%; $p<.004$) to drop out of treatment. Differences in treatment retention were not found for APD/non-APD subjects (33.3% vs. 25.7% dropout; ns).

Conclusions. The findings indicate that both antisocial and non-antisocial MM patients benefit from treatment. There is limited evidence that psychopathic patients represented a more severe form of antisocial subgroup than those qualifying for an APD diagnosis.

DISCUSSION

G. E. Woody

Antisocial personality disorder (APD) is a very serious problem among persons with substance use disorders. It is the one pre-existing disorder that has consistently been shown to serve as a risk factor for the development of substance use disorders. It is associated with an increased risk for HIV infection and an increased death rate, often attributable to violence or accidents. Most studies have found that persons with APD do not do as well in treatment as those without the disorder. However, many studies also show that APD is a heterogeneous category. Some indicate that there is a "treatment responsive" subgroup.

Dr. Cottler's study is one of the first that examines the relationship between adult antisocial behavior, drug use, and behaviors that increase the risk for HIV infection among women. She finds that women with AAB use more drugs than women without it. Over a period of 12 months she finds that those with AAB decrease injecting drug use but that they are less likely to decrease cocaine use than those without AAB. In contrast to the improvements in drug use and its associated risky behavior, she finds no significant decrease in risky sexual

behavior. She also finds that major depression is associated with better outcome. These data are very consistent with other studies which have shown that risky behavior associated with drug use is more amenable to change than risky sexual behavior, that persons with APD am less responsive to treatment, and that patients with both depression and APD have a better response to treatment than those with APD alone.

Dr. Crowley's data are very interesting and unique. The level of social problems displayed by his group of adolescents is stunning. The data offers an insight into a large group of very disordered children who ate in great need of help. These adolescents are obviously at very high risk for the later development of APD. The data presented indicate that large numbers of these children may respond to treatment, though the intensive therapy provided is often not available elsewhere. Additional work in this area, especially controlled studies of treatment outcome, strike me as very important. If left unattended, these adolescents are very likely to cause very serious problems for themselves and others as they become older.

Dr. Brooner's study adds a new dimension by suggesting that not only APD, but Axis II disorders in general are associated with a poorer response to treatment. Dr. Brooner's findings are definitely worth pursuing in other studies and with other populations; they probably apply to other disorders as well.

Dr. Alterman's study indicates that those with APD have more severe drug problems than those without APD, and that they improve with treatment. In this regard, his data are similar to those found in most other studies. His data differ, however, in finding that persons with APD improve at the same rate as those without the disorder. Most studies have found slower rates of improvement among persons with APD than those without it. It is uncertain why his data differ from that of others in this regard; perhaps his subjects represent more of the "treatment responsive" subgroup than other studies, perhaps earlier samples were unrepresentative of the overall program, or perhaps our treatment program is doing a better job with these patients than we did in the past !

In summary, these studies address a range of important issues surrounding APD and raise a number of interesting questions. How does APD influence outcome compared with other Axis II disorders? are adolescents with conduct disorders responsive to therapy and if so, what kind and for how long? how can we best treat persons with APD, especially in reducing their risk for HIV infection? These and other questions am important areas that should be addressed by future clinical studies.

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CARDIOVASCULAR EFFECTS OF COCAINE: UNDERLYING MECHANISMS

C. W. Schindler

High levels of cocaine-related emergency room admissions led to a renewed interest in the cardiovascular effects of cocaine in the late 1980's. As a result, a great deal has been learned about cocaine's effects on the cardiovascular system in recent years. The purpose of this symposium was to bring together researchers with a broad range of views on the mechanisms underlying the cardiovascular effects of cocaine. The work presented includes both animal and human research. Despite an overall decline in the abuse of cocaine in recent years, the continued high levels of emergency room admissions citing cocaine use gives added importance to the study of cocaine's cardiovascular effects.

OVERVIEW OF COCAINE'S CARDIOVASCULAR EFFECTS IN ANIMALS

R. D. Wilkerson

Hypertension, sinus tachycardia and elevated circulating levels of catecholamines are *sine qua non* of the acute actions of cocaine in humans. Both the pressor effect and the sinus tachycardia associated with cocaine administration appear to be catccholamine-mediated, since pretreatment with appropriate adrenergic antagonists has been shown to inhibit both actions. In addition to these global cardiovascular actions, cocaine has also been shown to affect the coronary circulation in humans. Although much has been written about cocaine-induced large epicardial coronary artery vasospasm and myocardial infarction, vasospasm has not been observed in patients who received cocaine during coronary arteriography. Indeed, only a modest reduction in the diameter of large coronary arteries was observed, without any evidence of mycocardial ischemia.

Animal models employed to study the cardiovascular actions of cocaine should, at a minimum, exhibit the three cardinal actions of cocaine described above for humans. To date, those actions of cocaine have been demonstrated in a number of species, including rats, dogs, rabbits and subhuman primates, but a requirement is that the animal be studied in the conscious, fully awake state. Numerous studies have shown that anesthesia or sedation significantly blunts all of the above described actions of cocaine. Indeed, when cocaine has been administered to anesthetized animals, a common finding has been a decrease, rather than an increase, in blood pressure, presumably a manifestation of the vasodilator effects mediated by the local anesthetic action of cocaine, which is not masked by increased adrenergic activity in this preparation.

In animal models which exhibit cocaine-induced cardiovascular responses similar to those observed in humans, the mechanism(s) of action of cocaine is still unclear. Some effects of cocaine, such as myocardial depression, are, undoubtedly, the result of the local anesthetic actions, but many questions surround the adrenergic actions of cocaine. Although there appears to be general agreement that circulating catecholamines are elevated by cocaine in conscious animal models, it is not clear whether this is merely the result of inhibition of peripheral neuronal monoamine uptake, or whether peripheral sympathetic tone is elevated by a central action of cocaine. If peripheral sympathetic tone is elevated, the relative roles of sympathetic neuronal stimulation and adrenal medullary catecholamine release are also unclear at this time. In this latter regard, recent studies, even in conscious animals, have demonstrated that the activity of specific sympathetic neurons innervating the heart and some regional vascular beds is inhibited after cocaine administration. To date, there has been no explanation for this apparent paradox; that is,

increased adrenergic activity, as evidenced by hypertension, tachycardia and increased circulating catecholamines, occurs in association with decreased peripheral sympathetic nerve activity.

PERIPHERAL MECHANISM'S IN COCAINE'S CARDIOVASCULAR EFFECTS

R. A. Gillis with F. E Kuhn, H. K. Erzouki and Y. M. Hernandez

Cocaine administration is associated with a number of cardiovascular changes including increases in arterial blood pressure (BP), heart rate (HR), rate-pressure product and coronary vasoconstriction. These changes are due to a cocaine-induced augmentation of sympathetic nervous system function. Currently, augmentation of sympathetic nervous system function produced by cocaine is thought to be due primarily to stimulation of sympathetic centers in the brain (central effect) and to inhibition of catecholamine uptake into post-ganglionic sympathetic nerve terminals (peripheral effect). It is our thesis that a peripheral effect can explain most of the sympathomimetic effects of the drug on the cardiovascular system. Evidence that cocaine-induced changes in cardiovascular function, particularly those that peak within 1 to 5 minutes after an i.v. bolus injection of the drug, are due to a peripheral effect is as follows: (1) the time course of action and dosage range of cocaine to potentiate neurally-released and injected norepinephrine (NE) on the heart follows that which increases BP, HR. rate-pressure product and coronary vasoconstriction; (2) cocaine given in i.v. doses that increase BP has no significant effect on spontaneously occurring cardiac sympathetic nerve activity; and (3) cocaine methiodide, a quaternary derivative of cocaine which is not able to cross the blood-brain barrier, given in equimolar doses to cocaine, produces quantitatively and qualitatively similar cardiovascular responses to cocaine. Cocaine methiodide has been shown to act peripherally to inhibit the uptake of NE into post-ganglionic sympathetic nerve terminals. Further evidence for a lack of central excitatory effect of cocaine on sympathetic centers are the findings that: (1) cocaine given into the cerebroventricles in doses that would reach the brain when given systemically in recreational doses has very little effect on BP and HR; (2) cocaine administered directly into the blood supply of the hindbrain produces no increase in BP, HR or sympathetic nerve activity; instead, decreases in BP and sympathetic nerve activity occur.

Two additional points about cocaine's actions on the peripheral sympathetic nervous system are that: (1) tachyphylaxis develops towards cocaine's potentiating effect on the heart, and (2) cocaine given in doses of 2 to 4 mg/kg i.v. can act at sympathetic ganglia to reduce post-ganglionic sympathetic nerve activity. Tachyphylaxis appears to be mediated in part by the interaction of synaptic accumulated NE on presynaptic alpha-2 adrenoceptors on post-ganglionic cardiac sympathetic nerves, while reduction of sympathetic ganglionic transmission appears to be mediated in part by the interaction of synaptic accumulated NE on ganglionic alpha-2 adrenoceptors. Based on our results we conclude that the mechanism for cocaine's sympathomimetic effect on cardiovascular function is unrelated to any excitatory effect of the drug on CNS centers, but is due primarily to the ability of the drug to inhibit uptake of catecholamines into post-ganglionic sympathetic nerve terminals.

PHARMACOLOGICAL MECHANISMS IN COCAINE'S CARDIOVASCULAR EFFECTS

C. W. Schindler

Cocaine has a diverse pharmacology. For example, cocaine produces potent blockade of the uptake of dopamine, norepinephrine and serotonin as well as local anesthetic effects. Our laboratory is interested in how this diverse pharmacology contributes to cocaine's

cardiovascular effects. In conscious squirrel monkeys, we have tested a variety of compounds as cocaine pretreatments. The squirrel monkey is a reliable model for cocaine's cardiovascular effects in that it mimics the human response to cocaine. Moderate to high doses of cocaine produce a sustained pressor effect and tachycardia. Both the non-selective α-adrenergic antagonist phentolamine and the selective α--1 antagonist prazosin completely block this pressor effect of cocaine. In contrast, the non-selective ß-adrenergic antagonist propranolol and the ß-1 selective antagonist atenolol completely block the tachycardia produced by cocaine. These results indicate the importance of α-1 and ß-1 adrenergic mechanisms in mediating the pressor and tachycardiac effects of cocaine respectively. While propranolol was effective as an antagonist of cocaine's tachycardiac effect, it simultaneously enhanced cocaine's pressor effect, thus making its utility as a treatment agent questionable. We found little support for a role of dopaminergic mechanisms in the hemodynamic effects of cocaine. Neither the dopamine D1 selective antagonist SCH 23390 nor the D2 selective antagonist haloperidol attenuated the pressor effects of cocaine and only haloperidol slightly attenuated the tachycardia following cocaine. We have also tested a variety of calcium channel antagonists In general, the calcium channel antagonists were able to only attenuate the cocaine-induced pressor response.

Toxicity to cocaine is often observed hours following its administration, pointing to a potential role of the cocaine metabolites. In an anesthetized rat preparation where the local anesthetic effects predominate, high doses of cocaine produce a depressor effect, bradycardia and QRS widening. Cocaethylene, a metabolite produced with co-administration of cocaine and ethanol,produced comparable effects. Unlike cocaine, norcocaine tended to increase blood pressure at higher doses while simultaneously widening QRS. The major cocaine metabolites benzolyecgonine and ecgonine methyl ester did not produce QRS widening, but did produce clear pressor effects. These results indicate that the cardiovascular effects of cocaine arc not necessarily mimicked by its metabolites and therefore these differing effects of the metabolites should be considered when evaluating cocaine's cardiovascular toxicity.

CARDIOVASCULAR EFFECTS OF COCAINE IN HUMANS

R. W. Foltin and M. W. Fischman

Studies in which experienced cocaine users receive cocaine under controlled laboratory conditions are an excellent source of data on the cardiovascular effects of cocaine. Studies with intranasal, intravenous. and smoked cocaine all show that under conditions simulating cocaine "bingeing," in which subjects are allowed to take cocaine repeatedly, heart rate (HR) generally returns to near baseline levels despite gradually increasing cocaine blood levels. In contrast, blood pressure (BP) has been shown to gradually increase when the dosing was i.n. and the time between injections approximately 35 minutes, although with the other routes of administration it generally mirrored the HR effect. The effects of repeated doses of smoked or i.v. cocaine were directly compared in 10 research volunteers who received the same smoked or i.v. cocaine dose twice with a 14 min interval between doses. When smoked and i.v. cocaine administration resulted in similar venous plasma levels, similar cardiovascular effects were observed, with minimal differences as a function of route of administration. The repeated-dose data suggest the rapid development of acute *(i.e.,* with session) tolerance to cocaine.

Cocaine is often self-administered in close temporal proximity to ethanol, marijuana or heroin. An unexpected cardiovascular interaction was observed in nine subjects who inhaled cocaine and drank ethanol-containing beverages. This combination resulted in HR increases that were significantly larger than observed with either drug alone, but produced similar or lower BP increases than that produced by cocaine alone. A single i.v. dose of either cocaine or morphine sulfate alone produced dose-dependent increases in peak HR and BP, but there were few instances in which the cardiovascular effects of

combinations of cocaine and morphine were significantly greater than those produced by cocaine alone. When i.v. cocaine was combined with smoked marijuana in seven male volunteers, combinations of cocaine and marijuana increased HR above levels seen with either drug alone. Increases in BP following combinations of cocaine and marijuana were equivalent to those produced by cocaine alone. These studies indicate that it is impossible to predict the cardiovascular effects of drug combinations based on the effects of either drug alone. HR was often increased substantially by drug combinations, while the effects of a drug combination on BP were often equal to that of one drug alone. Likewise, the interaction of potential treatment drugs with cocaine should be assessed under controlled settings prior to large scale treatment studies. When six research subjects were allowed to self-administer i.v. saline or cocaine (8, 16, or 32 mg) before and during a period of maintenance on desipramine there was a significant increase in baseline HR and BP during desipramine maintenance. Cocaine administration engendered increases in HR and BP above the desipramine-elevated baselines, suggesting that cocaine self-administration may have the potential for greater cardiovascular effects when patients are maintained on desipramine. Clearly, drug interactions can have unexpected cardiovascular effects, and laboratory studies provide a controlled setting for understanding and researching these interactions. Such data are necessary for the safe introduction of new pharmacotherapies for substance abuse treatment.

EFFECTS OF COCAINE ON VAGAL TONE IN HUMANS

D. B. Newlin

This research was conducted within the broad framework of our research looking for commonalities in the cardiovascular response pattern of drugs of abuse in humans. Based on empirical results with various abused substances, we hypothesized that acute cocaine would produce a distinct cardiovascular pattern consisting of tachycardia with a reduction of cardiac vagal tone. Vagal tone index is a noninvasive measure of parasympathetic inhibitory influences on the heart. It is derived using time series analysis from sequential R-wave to R-wave intervals of the ECG; it quantifies respiratory sinus arrhythmia, or heart rate variability at approximately 0.30 Hz. When tachycardia is associated with reduction in vagal tone, it implies that the increased heart rate is due to *withdrawal* of vagal chronotropic inhibition rather than due to active sympathetic activation. We have observed this characteristic pattern, to varying degrees, with a broad range of different drugs of abuse, including marijuana. nicotine. alcohol. methylphenidate, morphine: pentobarbitol and diazepam. These drugs are dramatically different pharmacologically, but are common in producing: 1) self-administration; 2) positive place-preference conditioning; 3) low dose locomotor activation effects; 4) reductions in cortical glucose utilization in neuroradiological studies in humans; 5) generally positive subjective effects; and 6) vagally-mediated tachycardia. We have been able to rule out this cardiovascular pattern with several nonabused or aversive drugs, including naloxone in opiate-dependent individuals, flumazenil (a benzodiazepine antagonist), and mCPP (a serotonergic drug that is generally dysphoric).

Cocaine (IV., 20 mg and 40 mg) and placebo (saline) were administered on separate days to 14 male residential volunteers with histories of cocaine abuse. Cocaine produced dose-dependent increases in heart rate. This effect was precisely mirrored by robust decreases in vagal tone index, as well as decreases in a lower frequency (approx. 0.10 Hz) heart rate rhythm associated with blood pressure homeostasis. Injection of saline *(i.e.,* cocaine cues) produced an initial 14 bpm increase in heart rate that had no significant vagal component. Vagal tone index and the lower frequency rhythm decreased approximately 2 to 2.5 log units in response to 40 mg cocaine, with a trough 9 to 14 minutes after I.V. administration. Therefore, cocaine led to a pronounced decrease in heart rate variability. The results indicate that cocaine-induced tachycardia has a strong parasympathetic component and appear to contradict the common assumption that tachycardia from cocaine is due primarily to sympathetic activation. It is consistent with the view that at

low to moderate doses in humans, cocaine increases heart rate due to withdrawal of vagal inhibition. Evidence indicates that vagal inhibition normally suppresses cardiac arrhythmias, and that profound release of vagal restraint can be arrhythmiagenic. Moreover, our results indicate that this effect is conditionable in the sense that cocaine placebo given to experienced users produced robust, but short-term, placebo and anticipatory responses in the same direction as the drug itself. The central neuroanatomical and neurochemical substrates that control this common cardiovascular pattern are not known.

DISCUSSION

H. R. Levin

The majority of cocaine-related emergency room visits and associated hospitalizations are due to cocaine's cardiovascular effects. Thus, a clear understanding of the physiologic mechanisms of these effects are necessary to develop specific treatments. In the papers presented above, the effects of cocaine on the peripheral and central nervous systems have been described. A functional understanding of these systems and their effects on autonomic tone are necessary in order to appreciate the clinical significance of these findings. For example, a lethal complication of cocaine use, sudden cardiac death (SCD), is a mutifactorial event that may be precipitated by cardiovascular, neurological and/or hematologic events. Though a sustained arrhythmia is probably the final event leading to SCD, these arrhythmias are caused by the combination of an abnormal substrate *(i.e.,* myocardium) and a trigger *(i.e.,* a premature ventricular contraction {PVC}). Abnormalities in autonomic tone can produce PVCs. In addition, changes in autonomic tone can alter the electrical properties of the myocardium, allowing a PVC to initiate a sustained arrhythmia. The cardiovascular effects described in the previous papers may lead to ischemia, myocardial infarction and fibrosis. These factors can also adversely alter the electrical properties of the myocardium, predisposing it to SCD. However, factors other than those previously mentioned may play a role in producing SCD. Although cocaine transiently decreases ventricular contractility, the prolonged decrease in ejection fraction from cocaine-induced peripheral vasoconstriction may result in vital organ hypoperfusion. Further, unwitnessed deaths assumed to be caused by SCD may in fact be the result of cocaine-induced seizures or malignant hyperthermia. Finally, cocaine may alter platelet aggregation increasing the risk of coronary thrombosis.

While an understanding of the cardiovascular effects of cocaine are important, there arc other important health problems associated with drug abuse such as AIDS, hepatitis and endocarditis which necessitate new treatment modalities. Similar to any other drug or device development, human research is needed to show the efficacy of a new treatment. Certainly, administration of cocaine to subjects carries a risk of adverse cardiac events. However, the doses used in human studies are well below average street doses and the trials are performed under controlled conditions that, if necessary, would allow prompt treatment of any adverse event. In addition, it should be noted that the cardiovascular changes noted during human studies are less than those observed during a clinical exercise stress test or with normal exercise. Thus, while human research may carry some risk, these trials may allow the development of treatments not only for the cardiovascular effects of cocaine, but the problem of cocaine addiction as well.

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FORUM ON DRUG RESEARCH WITH POLICY IMPLICATIONS

B. A. Rouse and J. H. Autry, III.

As part of the process by which science in general and drug research in particular can inform policy decisions, this Forum presented drug research of current policy significance, highlighted the importance of policy relevant research, and encouraged researchers to look for policy relevance in any research they undertake. The related policy issues discussed include drug detection, health policy approaches to drug abusers with infectious and/or contagious diseases (e.g., partner notification), criminal justice system approaches to drug abusers, and the assessment of the quality of care. Dr. Herb Kleber discussed the policy implications of the research presented and provided a wider perspective from which researchers in general can play a role in policy development.

The first presentation was on **"Analytical Challenges in the Chemical Diagnosis of Drug Exposure Using Urine, Blood, Saliva and Hair"** by Donna M. Bush and Edward J. Cone. Mandatory Guidelines for Federal Workplace Drug Testing Programs were issued on April 11, 1988. These implemented the National Laboratory Certification Program and became known as the "gold standard" for workplace drug testing. These Guidelines specified urine as the testing specimen and prescribed testing procedures for drugs of abuse, using two independent chemical tests: immunoassay and gas chromatography/mass spectrometry.

Because, in essence, drug test results are now evidentiary in nature and the consequences of a positive drug test in the workplace may include adverse personnel action, the accuracy and reliability of drug testing procedures must be paramount. This presentation examined the current state of knowledge concerning the pharmacological profile of drug actions and analytic studies of the testing process in urine, blood, saliva and hair. Each specimen is unique with its own properties which must be understood; each specimen tells us something new and different.

Blood is the specimen where pharmacokinetics of a drug can be measured. E.g., kinetics following cocaine smoking or IV administration are similar and reflect even absorption and elimination compared to nasal inhalation which shows uneven absorption characteristics. Blood may be useful in looking at performance impairment since blood reflects the amount of drug in the brain more closely that other specimens. Studies are needed to examine the relationships among drug administration route, blood concentrations, and performance impairment as well as studies of blood levels and pharmacokinetics. As far as research on saliva is concerned, not only is there a lack of data on levels of drug in saliva and effects on

behavioral performance, but saliva drug concentrations have been found to correlate poorly with plasma concentrations and pharmacological drug effects.

Although hair analysis appears to be an attractive alternative to urine drug testing because it may provide a long term window for drug detection, this is an area of new science with few controlled clinical studies. Observations of drug using populations show large concentrations of cocaine deposited in hair from cocaine ingestion, small to moderate amounts of cocaine from passive exposure, but only small concentrations of heroin from ingestion. The amount of drug deposited and/or detected may be affected by recency of drug use, hair color, chemical treatments, environmental contamination, and varying rates of hair growth on different parts of the body. Commercially available urine drug test kits have not been approved by the FDA for use with hair specimens.

In summary, analytical considerations (e.g., accuracy, precision, sensitivity, specificity, quality control, and interpretability of results) are well established for urine as the specimen, less well established for blood, and are in basic developmental stages for saliva and hair. The scientific basis of drug testing specimens, rather than expediency, must be carefully considered in policy decisions and applications.

"HIV Transmission Models as Epidemiologic Tools for Evaluating the Consequences of Partner Notification Programs" by Sally Blower, Ph.D., University of California, Berkeley, CA was presented next. Notification of sex partners of HIV+ persons identified through counseling and testing programs has been conducted in some states to curtail the HIV epidemic. Mandatory notification programs to be implemented by state health departments throughout the country has been proposed. Research into the effects of partner notification, however, is scant. Unknown are the degree notified individuals are likely to reduce their risk behavior, the degree HIV seroconversion in HIV discordant partnerships is prevented, and the adverse consequences of partner notification programs (PNP). E.g., an HIV+ woman tested as part of her prenatal care may be required to inform her injection drug using partner which may result in violence toward the woman or dissolution of the relationship. It is also possible that there may be strong community reactions to mandatory PNP resulting in avoidance of contacts with health institutions.

This presentation discussed how models of the transmission dynamics of HIV could be used to evaluate PNP as an epidemic intervention strategy. HIV epidemics are complex non-linear systems characterized by specific transmission processes, multiple linked risk groups with different risk levels of acquiring HIV, interaction between population and individual level processes, risk behavior changes with both direct and indirect effects, and extremely long and variable incubation time.

Three different approaches for modeling PNP were discussed: scenario analyses, basic reproductive rate calculations, and sensitivity analysis. Results from a simulation model of the HIV epidemic for New York City were presented. This subgroup transmission model included HIV transmission probabilities for sexual and needle-sharing exposure with stranger or buddy as well as the population mixing

patterns which result in HIV exposure contacts. It took into account a bridge population (non-injecting heterosexual partners of IDU), infection states (susceptible, HIV seropositive, AIDS), differential non-AIDS mortality rates, and gender differences in injecting and sexual behavior. Findings included the following: The degree of sexual mixing that occurs between injecting drug users and non injectors is critical in determining the future number of AIDS cases in women and pediatric AIDS in NYC. Prediction precision in the future number of AIDS cases for NYC was low; but that imprecision was due to the estimation uncertainty of only a few biological-behavioral parameters. These key variables were the heterosexual transmission efficiencies per partnership (i.e., the average probability that a susceptible individual will acquire HIV during a heterosexual partnership given that the partner is infected), the adult incubation time, and the vertical transmission efficiency.

In summary, currently, the only way that HIV epidemics may be controlled is by risk behavior reduction. To evaluate the significance of behavioral changes, it is not sufficient to know only the type, magnitude, and timing of the behavioral change. The seroprevalence level at which the behavior change occurs and the prevailing mixing patterns of the various at-risk groups are also important. Partner notification programs have been suggested as a potential epidemic control measure. Before such programs become public policy it is necessary to assess their potential efficacy, ethical issues, and acceptability to target communities. Policy decisions should take into account the potential for varied impact on population subgroups; mathematical models are useful to preview these impacts.

Next was **"Outcomes of an Experimental Intervention Directed to Drug Abusing Parolees"** by David Nurco, T.E. Hanlon, R.W. Bateman, T.W. Kinlock, and E. Toledano, University of Maryland School of Medicine and Friends Medical Science Research Center, Baltimore, MD. An experimental intervention designed to reduce drug use and crime by male and female parolees with a history of narcotics or cocaine use was presented. The experimental project offered intensive social support, case management with social services job bank, client advocacy and weekly urinalysis for 12 months post-prison release. The two control conditions were weekly urinalysis at the project site and routine parole supervision (agent visits and random urinalysis). Parole record reviews indicated that the experimental group had the lowest number of arrest warrants, rearrests, and reincarcerations 6 months post-release; the next lowest was among the control group with weekly urinalysis. The most predictive variables of counselor-rated lack of treatment success were being male, other family members involved in crime, and younger age at first crime.

The importance and difficulty in correctly identifying drug abusers within the correctional system and of matching these clients to treatment were addressed. Newly available instruments to help include the Drug Offenders Profile and Referral Assessment (DOPERS) which provides more specific information on the relationship of drug involvement to criminality, the Offender Profile Index which serves as a preliminary triage function, and the "heuristic" approach to drug abuse classification and treatment planning used at Patuxent in Maryland to guide drug abuse intervention efforts.

Based on this research, the following were recommended: continuity between rehabilitative efforts in correctional settings and treatment efforts in the community; drug testing prior to a client's release from custody because some inmates have access to drugs while incarcerated; escorting parolees to their treatment clinic rather than relying on their own initiative in making the necessary clinical contact; consistency in disciplinary actions for infractions occurring under parole conditions; periodic transmittal of treatment progress information (e.g., treatment compliance, accomplishment of treatment goals, and evaluation of personal and community adjustment) from treatment programs to parole officers to be considered in determining the parole status of individuals at any given point in time; and, finally, the use of a progressive series of intermediate sanctions to work on reducing deviance through more intensive clinical efforts and the aggressive use of significant others and available community resources.

The final presentation was **"National Study of Drug Treatment Quality Standards and Assessment"** by Beatrice A. Rouse, Ph.D., SAMHSA and Richard W. Scalenghe, JCAHO. The current emphasis on cost-effectiveness and cost containment in health care reform indicates the importance of determining, measuring, and monitoring the quality of care given. Two studies were presented which were conducted to determine the formal mechanisms in the current delivery system used to promote drug abuse treatment quality improvement. Study #1 was a descriptive national study of the licensing and credentialing standards and requirements for drug abuse counselors and programs as of 1991. Data were extracted from the latest available legislation and state regulations. The majority of states (39 plus the District of Columbia) had voluntary certification requirements for drug abuse/chemical dependency counselors. While States with mandatory certification were more likely to require drug abuse education or training and those with voluntary certification were more likely to accept relevant experience, the differences were not statistically significant. Ten states had drug abuse specific requirements for noncounselor drug treatment practitioners. Twenty states accepted JCAHO or CARF accreditation as full or partial compliance for state licensing or certification. Several additional states also accepted such accreditation for meeting State insurance requirements.

Study #2 was an indepth case study of formal quality assessment programs in selected drug abuse treatment programs from a variety of modalities, settings, and funding sources. Philosophical differences were noted between the two current major approaches to setting, evaluating, and monitoring standards of care: Quality Assurance is a more problem oriented approach focussing on identifying and monitoring sentinel indicators such as suicide, violence, disappearance of medications, etc. and comparing performance against some set of standards. In contrast, Continuous Quality Improvement is more system-oriented with the emphasis on aggregate data such as mean time to relapse, attrition rates, etc. Proponents feel that this latter perspective moves the standards and quality of care constantly upward rather than focussing on some minimum, static standard which eventually tends to become the goal. The standards, assessment and improvement procedures, and staff involvement were described for 5 exemplary programs. In addition, examples of the different quality assessment approaches, data elements,

and data sources were presented for discharge planning and for examining cases that left against program advice.

In summary, the State system for setting treatment standards consisted of a combination of standards, guidelines, policies, laws, and regulations. Also, States differed in the level of specificity of their requirements. Often, the most effective control mechanisms imposed by the States were those directly related to funding decisions. In regard to formal quality assessment mechanisms by individual programs, no clear trend emerged as to their design or implementation. Equally successful programs varied vastly in their approaches. In general, treatment was monitored in a systematic way with reasonable criteria. Both setting and evaluating appropriate drug treatment quality and continuous improvement in care given also requires attention to the organizational structure under which treatment is provided as well as to the process and outcome of care. Designated quality assurance and improvement programs are only part of the effort to improve treatment for substance abuse. Other aspects include developing patient placement criteria and clinical guidelines; targeting services for special needs (e.g., pregnancy); matching the treatment to the patients; and formally implementing relapse prevention and after care services.

The discussant was Dr. Herbert D. Kleber who shared some of his experiences with data needs for policy decisions while Deputy Director for Demand Reduction at the Office of National Drug Control Policy. He indicated that we still need timely and accurate research on such epidemiologic issues as how many people need what kinds of treatment, how many users get addicted, and how to reliably and validly measure drug use and addiction. Treatment issues included the natural history of people who enter the treatment system, patient/treatment matching, and the critical factors predictive of successful treatment outcome. Dr. Kleber indicated that the public expectations for successful substance abuse treatment included decreased use of medical services, elimination of criminal activities, employment, reduced family disruption, and the maintenance of abstinence from drug use. Research needs regarding prevention included protective factors from drug abuse, effective prevention for different segments of the adolescent population, and how to do prevention in the declining phase of an epidemic. Finally, he outlined some needed policy changes which included better targeted preventive efforts which emphasized protective factors as well as risk factors, focus on community efforts, the use of treatment as an alternative to prison, expanded treatment capacity, and comprehensive substance abuse care to include coexisting conditions such as HIV and TB.

Taken as a whole, the studies presented show the importance of research and its application to policy. Research not only can but should inform policy and contribute to better policy formulation. The application of research to policy development is an important part of the continuum of basic, preclinical, clinical, and applied research.

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DRUGS OF ABUSE AND IMMUNOSUPPRESSION

M. J. Kreek, T. Eisenstein, H. Friedman, P. Peterson, L. Soderberg and L. S. Brown

OPIATES AND THE IMMUNE SYSTEM

T. K. Eisenstein, T. J. Rogers. J. L. Bussiere, M. Rojhavin, I. Szabo, J. J. Meissler, S. M. Belkowski, E. B. Geller and M. W. Adler

The central question which our research addresses is whether opioids can act as immunomodulators. Our studies have shown that opioids are immunosuppressive when given in vivo or in vitro. Our published work demonstrates that the μ and κ agonists inhibit the in vitro secondary response to sheep red blood cells (SRBCs) of murine splenocytes. Suppression by morphine is blocked by pretreatment with naloxone, and that caused by the κ agonist, II-50,488H. is blocked by norbinaltorphimine (nor-BNI). New data show that the δ agonists, DPDPE and D-Ala^2,Glu^4 -deltorphin, am also suppressive in this assay. Use of selective δ_1 and δ_2 antagonists showed that both DPDPE and deltorphin were inhibited by cys-4-deltorphin, but not by DALCE, supporting a role for $\delta_2$but not δ_1, receptors in immunosuppresssion.

In another type of experimental paradigm, groups of mice were implanted with a 75-mg morphine pellet, with a naltrexone pellet, or with both types of pellets. Sham-operated or normal animals served as controls. Morphine treatment inhibited the primary in vitro plaque-forming cell response to the SRBCs in all mouse strains tested, and simultaneous implantation of a naltrexone pellet blocked suppression in mice of the C3H lineage. Addition of normal macrophages to morphine-suppressed cultures restored responses. Addition of recombinant interleukin-1 (IL-1), IL-6 or interferon-γ also restored responses in a dose-dependent fashion, whereas IL-2. IL-4, and IL-5 were not effective in reversing suppression. Morphine pellet-implanted mice had reduced spleen sizes. The immunosuppression was evident whether the number of antibody forming cells was calculated on a per spleen basis or per 10^{-7} cells. The immunosuppression may have resulted either from a relative decrease in the numbers of macrophages following morphine administration, or from decreased functional capacity of individual macrophages. Experiments were carried out to test the capacity of normal or morphine-treated murine resident peritoneal cells to phagocytize the yeast, Candida albicans When morphine was administered in vivo by pellet implantation, periotoneal exudate cells showed approximately a 40 percent reduction in Phagocytic Index (number of yeasts per macrophage based on microscopic examination of 200 to 400 cells), at a particle to macrophage ratio of 10: 1, and a 30 min period of phagocytosis. Phagocytosis was not depressed in mice receiving morphine and naltrexone pellets. Addition of morphine in vitro resulted in 40 percent depression of phagocytosis at 10^{-8}M. The effect of 10-6M morphine was blocked by naltrexone (10^{-8} M) and CTAP (10^{-6} M). but not by nor-BNI or naltrindole. indicating the action of the agonist was through a μ receptor. It was also found that DAMGO, U50 and DPDPE added to spleen cells *in vitro* depressed phagocytic function, and the action of these drugs was blocked by CTAP, nor-BNI. and naltrindole, respectively, in a dose-dependent fashion. These results support the conclusion that activation of μ, κ or δ opioid receptors decreases macrophage function. Additional support for this conclusion comes from studies on the macrophage cell line, P388Dl. U50 was found to inhibit LPS-triggered secretion of IL-1 as assessed by bioassay and by Northern blotting, and the inhibition was blocked by nor-BNI. In summary, immunosuppression can occur via μ, κ or δ opioid

receptors, as demonstrated when receptor-selective agonists and antagonists are administered in vivo or in vitro. Macrophages appear to be a prime target of opioid activity. (Temple University, Philadelphia, PA).

MARIJUANA AND ITS PSYCHOACTIVE COMPONENTS AS IMMUNOMODULATORS

H. Friedman

Marijuana and/or its psychoactive component, *i.e.,* tetrahydrocannabinol (THC), is known to affect the immune response system. Detailed studies have shown that chronic exposure of mice or rats to marijuana or its active components suppresses antibody formation, cellular immunity and/or lymphocyte blastogenesis. The marked immunosuppression has been attributed to direct THC effects. Studies in this laboratory have shown that antibody formation *in vitro* by mouse lymphoid cells are suppressed when spleens are obtained from animals injected with THC or metabolic products of this drug, such as 11-OH-THC. We have also found that these components of marijuana affect metabolism of lymphoid cells including protein kinase C activity, arachidonic acid metabolism, as well as calcium metabolism. Recently, we have also shown that cytokines, important as molecular signals by which cells communicate, are markedly affected by cannabinoids. For example, interferon production is suppressed, as well as production of IL-2 and TG cells activated to produce cytokines by a variety of stimulators. However, we have also found that THC, rather than being suppressive, enhances the production of pro-inflammatory cytokines and this appears related to increased susceptibility of cannabinoid-treated individuals to opportunistic micro-organisms. It has been shown that THC results in heightened susceptibility of mice to infection with a bacterium such as Listeria or to a virus-like herpes virus. Recently, we found that mice treated with THC have altered susceptibility to infection by Legionella pneumophila, an opportunistic intracellular bacterium which infects primarily macrophages. THC induced marked alterations in susceptibility of macrophages *in vitro* to these bacteria. Furthermore, mice infected with sublethal numbers of Legionella did not develop immunity to a secondary challenge infection with larger numbers of the bacteria. as occurs in non-treated animals. Furthermore, we found that mice infected with sublethal numbers of Legionella develop an acute death syndrome, similar to toxic shock death, when treated one day before and one day after infection with THC. Serum samples from such THC-treated animals showed increased levels of the acute phase cytokines, TNF and IL-6, which have been related to toxic shock death. Treatment of the mice with a single injection of a monoclonal antibody to TNF or IL-6. or a mixture of both. one hr prior to the second THC injection resulted in complete inhibition of the toxic shock-like' death. A marked fluctuation in blood granulocytes levels also occurred after THC injection, supporting the likelihood that the acute phase cytokine production was related to THC induced toxic shock-like death after sublethal infection with Legionella. Thus, results obtained in this laboratory have shown a relationship between the detrimental effects of THC on the immune response and heightened susceptibility to microbial infection and this appears to be mediated by the effects of the drug on cytokine production.
(University of Southern Florida, Tampa, FL)

MORPHINE AND COCAINE: MODULATORS OF IMMUNE SYSTEM COMMUNICATION

P. Peterson, T. Molitor and C. Chao

The mechanisms of opiate- and cocaine-induced immunomodulation involve both indiect effects (via central nervous system [CNS]- neuroendocrine pathways) and direct actions on cells of the immune system. While considerable understanding exists regarding the effects of these drugs on communication signals within the CNS (*i.e.,* neurotransmitters), little is known of the influence of opiates or cocaine on cytokines, the messenger molecules of the

immune system (*i.e.,"immunotransmitters").* Studies in our laboratories suggest that modulation of cytokine production may explain a number of the actions of morphine and cocaine on immune cells. The concept of morphine and cocaine as modulators of cytokine production arose from studies of the effects of these drugs both on human peripheral blood mononuclear cells (PBMC) in culture and on the pathogenesis of swine herpes virus (SHV)-1 infection in morphine-dependent pigs. Of relevance to the hypothesis that drugs of abuse act as cofactors in AIDS, we found that morphine and cocaine amplified the replication of HIV-I in PBMC cultures stimulated with cytomegalovirus. Increased production of two cytokines, transforming growth factor-ß and tumor necrosis factor (TNF)-α, by morphine- or cocaine exposed PBMC played in a central role in this *in vitro* phenomenon. On the other hand, morphine was found to markedly suppress the production of another cytokine, interleukin-1, by porcine alveolar macrophages in both an *in vitro* culture system and *in vivo*. This morphine-induced effect could explain, in part, the increased pathogenesis of SHV-1 pneumonia in morphine-dependent pigs. Interestingly, morphine-dependent pigs had a decreased severity of SHV-1 induced CNS disease. The latter observation contributed to our current interest in the immunomodulatory effects of morphine within the brain. Because the brain is a key target organ for morphine and cocaine as well as for neurotropic viruses. such as HIV-l and SHV-1, we have initiated studies of the immunomodulatory effect of morphine or microglia and astrocytes. These glial cells am known to serve many immune functions within the brain. Using primary murine microglial cell cultures, we determined that morphine potentiates the production of TNF-α by these macrophages. In an *in vitro* model of HIV-l infection of the human brain, we have found that morphine amplifies the expression of HIV-l via a mechanism involving increased glial cell production of TNF-α. These findings suggest that in addition to altering neurotransmission within the CNS, opioids may also function as modulators of immune cell communication within the brain (Hennepin County Medical Center, Minneapolis, MN)

IMMUNOTOXICITY OF NITRITE INHALANTS

L. Soderberg

Amyl, butyl and isobutyl nitrite are representative of a group of nitrite inhalants commonly called "poppers." These inhalants have been very popular among male homosexuals and to a lesser extent among adolescents. Inhalation of these compounds causes vasodilation, producing a "high", and also causes relaxation of smooth muscles, facilitating anal sex. Epidemiological studies have reported that, independent of other risk factors, heavy abuse of nitrite inhalants is a risk factor for HIV seropositivity and for Kaposi's sarcoma among AIDS patients. While it has been suggested that nitrite inhalant abuse is simply a marker of risky behavior, nitrites could facilitate infectious disease or tumor growth by depressing immune responses. Inhalation exposure of mice to isobutyl nitrite impairs both antibody and cell-mediated immune responses. Antibody production is particularly important in controlling extracellular bacterial infections. Exposure of C57B1/6 mice to 900 ppm isobutyl nitrite for 45 min/day for 14 days resulted in reductions of T-dependent antibody induction of up to 70 percent. Cell-mediated immunity is important for protection from viral infections and from growth of tumor cells. Two distinct cell-mediated immune mechanisms were impaired by exposure to the inhalant. Cytotoxic T cell and macrophage mediated tumoricidal activities were reduced by 36 percent and 59 percent, respectively, following immunotoxic exposure. Natural killer cell mediated cytotoxicity, on the other hand, was not affected. While the immunotoxicity seemed to preferentially affect T cell-dependent functions, the apparent targets of toxic activity were accessory cells, presumably macrophages. T-independent antibody responses and B cell mitogenic responses were not altered by the exposure, suggesting that B cells were not directly affected. In unseparated spleen cell cultures, T cell responses to allogeneic and mitogenic stimulation were inhibited. However, exposure to the inhalant did not affect T cell production of IL-2 or T cell expression of CD25, the IL-2 receptor beta chain. Purified T cells from exposed mice responded normally to mitogenic stimulation when untreated accessory cells were used,

suggesting that T cell functions were affected directly. Moreover, the ability of accessory cells from exposed mice to support the proliferation of control T cells was reduced by half. (University of Arkansas, Little Rock, AR)

CD4 DIFFERENCES BETWEEN HIV-INFECTED IDUs AND NON-IDUs

L. S. Brown

The use of the CD4 cell count in AIDS has provided an opportunity to discuss the merits and drawbacks of surrogate markers in general and the CD4 marker in particular. In research, reliable markers allow the development of useful information about the natural history of a disease and provide a means to assess the utility (safety and efficacy) of potential therapeutic interventions to modify the natural history of a disease. In medical care, surrogate markers provide an important clinical tool for diagnosis, assessing the efficacy for therapeutic interventions and determining the appropriate timing for prophylactic and therapeutic options. There are generally accepted criteria for surrogate markers. The surrogate should be biologically plausible and its presence/absence should be different among persons with and without the disease in question. The surrogate marker should correlate with important clinical disease progression events or endpoints. Useful surrogate markers show greater improvement in persons with treated disorders than untreated persons. In many natural history studies, injecting drug users (IDUs) experience a different range of opportunistic infections than other HIV infected populations, yet survival rates are similar. Many of these studies reveal higher CD4 count levels among IDUs as compared to other HIV infected populations. There are many reasons why the application of these markers in HIV disease may differ among various subpopulations. Reasons include diurnal mechanisms that affect CD4 distribution, age, viral load or replicating status of the virus, and concurrent immunomodulating disorders or therapies. Other factors include different measurement techniques or differences in the utilization of the measurement techniques. These findings suggest the need for two types of investigations to determine if clinically important differences in CD4 cell counts exist among subpopulations at risk for HIV infection: 1) comparative studies of CD4 cell count distribution among various non-HIV infected subpopulations; and 2) comparative studies of the CD4 levels when important clinical events first occur among various HIV infected populations. In both types of studies, there is a need to include IDUs, especially given the increasing impact of drug use among AIDS cases in the United States. (Addiction Research and Treatment Corporation, Brooklyn, New York and Department of Medicine, Harlem Hospital and the College of Physicians and Surgeons, Columbia University, New York, New York)

DISCUSSANT

M. J. Kreek

As discussed by the symposum participants, opiates, marijuana, cocaine, nitrite inhalants, and also alcohol may all significantly affect the immune system. Some of these agents may act as immune modulators; others may frankly disrupt the immune system. Of particular interest are the opiate effects on immune function, since such findings related to opiate effects first suggested that the endogenous opioids, the enkephalins, dynorphins, and endorphins, may play a role in the normal physiological modulation of immune function, and in addition. may impact upon immune function in an atypical way in the setting of pathological states. A great deal of research evidence has been forthcoming to document such effects of the endogenous opioid system on specific indices of immune function. It is provocative to suggest that other endogenous ligands which bind to the recently defined marijuana receptors may also serve as immune modulators. In all studies of the effects of drugs of abuse and also potential treatment agents on immune function, it is critical to consider first what constitutes these effects, that is. which cellular or humoral components of the immune system is affected, and, if more than one component is affected, what may be

the impact on the integrated immune system. Secondly, the question of whether any observed immune effects are direct effects, that is, the drug acting directly on cellular or humeral components, or indirect effects, for instance, by the drug altering endocrine and neuroendocrine function, must be addressed.

Finally, it has been well documented that there are profound species differences, both with respect to the effects of drugs of abuse on immune function, and on other systems, which may in turn alter immune function. Therefore, although many extraordinarily exciting findings may be made in whole animal models, as well as various in vitro systems, ultimately, the question of whether or not similar changes are observed in human drug abusers must be addressed. In man, normalization of neuroendocrine function occurs during steady dose, steady state methadone maintenance treatment of former heroin addicts. The normalization of immune function in a long-term methadone maintained patient may in fact be due to normalization of neuroendocrine function as seen in that setting. (The Rockefeller University, New York, New York)

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HALLUCINOGENIC AGENTS: DRUGS OF ABUSE AS NEUROCHEMICAL TOOLS

F. I. Carroll, R. A. Glennon, M. R Johnson, M. Teitler and D. M. Zimmerman

The purpose of this mini-symposium was to popularize the notion that drugs of abuse can serve as valid research tools to investigate various neurochemical mechanisms and may also act as templates for the design of novel therapeutically useful agents. Classical hallucinogens, for example, are becoming widely used to investigate serotonin receptors and to identify novel 5-HT2 antagonists. A series of N-substituted trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines have proven to be useful in the development of novel opioid antagonists and in the characterization of antagonist pharmacophores for the opioid receptors. Several classical and nonclassical cannabinoids were used to characterize the cannabinoid receptor and are continuing to be used to further understand mechanism of action of this class of compounds. Similarly, cocaine analogs are serving as biochemical probes to study the mechanism of action of psychostimulants. Some of the mom recent discoveries are summarized in the following sections.

CLASSICAL HALLUCINOGENS

Hallucinogenic agents are one of the oldest known classes of drugs, and the ready availability of these agents constitutes a significant and long-standing abuse problem. Lately, there has been a resurgence of interest in the use of classical hallucinogens and structurally-related designer drugs. Classical hallucinogens fall into two broad categories: (1) phenalkylamines such as the phenethylamine mescaline and the phenylisopropylamines, DOM, DOB, and DOI; and (2) indolealkylamines such as the N,N-dialkyltryptamine, DMT, the ergoline. (+)-LSD, and the ß-carboline harmaline. Although serotonin (5-HT) had been implicated as playing a role in the mechanism of action of these agents since its discovery in the late 1940s. it was not until 40 years later that evidence was provided for the involvement of 5-HT2 receptors (Glennon *et al.,* 1993). However, with the subsequent discovery of 5-HT1C receptors (Pazos *et al.,* 1984) came the finding that classical hallucinogens possess affinity for both 5-HT2 (now 5-HT2A) and 5-HTlC (now 5-HT2C) receptors, and that hallucinogenic potency was significantly correlated with receptor affinity in both cases (reviewed; Glennon 1993).

[^{3}H](+)-LSD was one of the early nonselective radioligands used to label 5-HT receptors. [^{3}H]DOB and [^{125}I]DOI are significantly more selective than [^{3}H](+)LSD and display high affinity for 5-HT1C/5-HT2 receptors. Unlike the standard 5-HTlC/5-HT2 antagonist radioligand [^{3}H]ketanserin, the two radiolabeled phenylisopropylamine hallucinogens appear to label the agonist high-affinity state of 5-HT2 receptors (Lyon *et al.,* 1987). With the recent cloning of more than a dozen different subpopulations of 5-HT receptors, phenylisopropylamine hallucinogens (which typically are more selective than the indolealkylamine hallucinogens) are being employed to aid in the classification of these receptors. These radioligands are also being used in autoradiographic mapping of certain 5-HT receptors *(e.g.,* Appel *et al.,* 1990).

Phenylisopropylamine hallucinogens exert stimulus control of behavior and can serve as effective training drugs in drug discrimination studies involving rats. Using DOM as a training drug, there is a significant correlation ($r > 0.9$) between stimulus generalization ED_{50} values and (1) human hallucinogenic potencies and (2) 5-HT2 receptor affinities for a large number of classical hallucinogens. Hallucinogen-trained animals are also being used to identify novel .5-HT1C/5-HT2 antagonists (which likely possess antidepressant,

neuroleptic, anxiolytic, and cardiovascular activity) and to investigate the stimulus properties of novel designer drugs.

USE OF TRANS-3,4-DIMETHYL-4-ARYLPIPERDINES AS SELECTIVE OPIOID ANTAGONISTS AND THERAPEUTIC AGENTS

N-Substituted-trans-3,4-dimethyl-4-(3-hydroxyphenyl)-piperidines represent a unique series of pure opioid antagonists (Zimmerman *et al.,* 1978). Prior to their discovery, opioid antagonists were generally N-allyl or N-methylcyclopropyl analogs of morphine and other multicyclic morphine-like agonists. The opioid antagonist activity in these 4-arylpiperidines is most importantly a consequence of 3-alkyl substitution of the piperidine ring. Earlier structure activity relationship studies had shown that affinities for opioid receptors depended largely on the nitrogen substituent. Further SAR studies within this series have now been pursued for the development of selective opioid antagonists as pharmacological probes and therapeutic agents. Alterations of the N-substituent led to the discovery of LY255582, an opioid antagonist with marked appetite suppressant properties (Shaw *et al.,* 1991). and recently to LY246736, a peripherally selective mu receptor antagonist with good activity following oral administration. These SAR studies also led to the discovery of many highly potent opioid receptor antagonists with only limited receptor selectivity. Importantly, no N-substituted derivative possessed detectable opioid agonist activity. Thus, this work confirms the pure opioid antagonist nature of the trans-3,4-dimethyl-4-arylpiperidine nucleus, and also suggests that additional SAR studies within this series could lead to the discovery of highly selective opioid receptor antagonists.

CANNABINOIDS

Consroe and Sandyk have written that the second modem era of cannabinoid research began in 1987 when Howlett and her colleagues at St. Louis University Medical School and Pfizer announced the discovery of the; cannabinoid receptor in rat brain. The foundation for the neurochemical tools they employed was laid in 1974 by Everette May and his group at the NIH who made the discovery that 9ß-hydroxyhexahydrocannabinol (HHC) was equipotent to morphine in the hot plate test. This was the first evidence that analgesia was a structurally dissociable feature of the cannabinoid molecule.

Johnson and Milne at Pfizer made a remarkable structural observation ("The Prostaglandin Overlap Hypothesis") linking the key structural features of HCC to the blockade of a prostaglandin E sensitive adenylate cyclase proposed in Collier's Hypothesis for the mechanism of action of morphine. Guided by this structural hypothesis, Johnson, Melvin, and their coworkers at Pfizer synthesized hundreds of "classical" tricyclics *(e.g.,* levonantralol) and "non-classical" bicyclic structures *(e.g.,* CP-55,940 and CP-55,244) over the next eight years. This work culminated in the medicinal chemical conceptualization of the cannabinoid receptor, "The findings of exceptional potency in the microgram/kilogram range, retained spectra of activity despite extremes of structural elaboration and simplification, regio-, stereo-, and enantiospecificity of action and indirect effects on multiple biochemical systems mandate a novel site of action involving a distinct neurotransmitter system."

The neurochemical tools and ligands developed by the Pfizer group were used by Howlett to elucidate a biochemical model of cannabinoid action by demonstrating that the N18TG2 neuroblastoma cell in culture provided a suitable model system for the study of cannabinoids at the cellular level. She showed that the inhibition of adenylate cyclase by both the classical and non-classical cannabinoids was (1) cell-type specific; (2) rapid and reversible; (3) mediated by Gi; (4) dose-dependent in nM range; (5) stereoselective; (6) NOT due to adrenergic. muscarinic, or opioid receptors; and (7) correlated with biological activity. Having established a biochemical correlate for the cannabinoid receptor, Howlett used the new neurochemical tool [^{3}H]-CP-55,940 and the same ligands

to demonstrate CP-55,940 receptor binding that was of high affinity (K_i = 68 pM). enantioselective, with rapid (<45 min) and reversible kinetics. Receptor binding correlated with functional models of analgesia (r > 0.9) and inhibition of adenylate cyclase (r > 0.9). Herkenham and coworkers extended the work of Howlett to a slice assay and were able to provide a complete neuroanatomical picture of cannabinoid receptor binding across species (rat/monkey/human). These neuroanatomical distributions. in turn, allowed Linda Matsuda in Brownstein's lab at the NIH to realize they had cloned the receptor (rat). More recently, the human receptor has been cloned. Currently, Kenner Rice and his group at NIDDK are preparing affinity ligands of CP-55,244 and WIN-55,212 to be used in searching for receptor subtypes, isolating and purifying drug receptors, studying physiological functions of drug receptors, producing antibodies of drugs, and producing anti-idiotypic antibodies to purify receptors and identify gene products.

These and other neurochemical tools will be necessary to further understand the role of the cannabinoid receptor and various mechanisms of action in order to answer the therapeutically relevant question, "Can we separate the traditional activities of cannabinoids (*i.e.,* specific agonists)?" To do this, we must find if there are physiologically relevant receptor subtypes. The availability of binding assays and affinity ligands from the three structurally distinct types of cannabinoid ligands (classic, *e.g.,* HU-210; non-classic, *e.g.,* 55,940 and 55,244; alkylamino indole, e.g., WIN-55,212) provides a good starting point for this search. The major neurochemical tool still lacking is a functional cannabinoid antagonist which would provide mechanistic insight as well as having potential therapeutic *(e.g.,* Parkinsonism) properties. At least three chemically distinct endogenous substances have been isolated (Mechoulam, Howlett, Childers). Full structural and functional characterization of all three of these may provide insight into the largely theoretical issue-Why does man have a receptor in his brain for psychoactive plant extracts? as well as providing new chemical templates for the design of selective cannabinoid therapeutants.

COCAINE: A STRUCTURE ACTIVITY RELATIONSHIP STUDY

Cocaine has several sites of action in the central nervous system; however, it is the site associated with the dopamine transporter that has been implicated in the reinforcing properties of cocaine (Kuhar *et al.,* 1991; Ritz *et al.,* 1987).

To obtain information relating the structural features of cocaine which lead to potent and selective binding at the cocaine site on the dopamine transporter, we examined the effects of variations of 2-carbomethoxy, 3-benzoyloxy, and N-methyl substituents as well as the stereochemistry of the system of cocaine on binding affinity at the dopamine transporter.

First of all, the cocaine binding site at the dopamine transporter was found to be stereo-selective (Carroll *et al.,* 1991b).

Removal of the N-methyl group of cocaine to give norcocaine resulted in only a small reduction in affinity. In contrast, the addition of a second N-methyl group to cocaine to give cocaine methiodide as well as the acetylation of norcocaine to give N-nor-N-acetyl-cocaine (RTI-54) yielded compounds with very low or no affinity for the transporter. N-Nor-N-benzyl cocaine (RTI-36) was about 6-times less potent than cocaine (Abraham *et al.,* 1992).

The most potent compounds in binding and behavioral studies repotted before our studies began were 3ß-phenyltropan-2ß-carboxylic acid methyl ester (WIN-35,065-2) and 3ß-(p-fluorophenyl)tropan-2ßcarboxylic acid methyl ester (WIN-35,428). the so-called "WIN compounds," reported by Clarke and coworkers (Clarke *et al.,* 1973) In order to further develop the pharmacophore model for the cocaine binding site, we synthesized ten new 4'-substituted phenyl analogs of WIN 35,065-2. We found that the p-chloro (RTI-31). p-bromo (RTI-51). p-iodo (RTI-55), and p-methyl (RTI-32) analogs were 13- to 20-times more potent than WIN 35,065-2. The log ($1/IC_{50}$) values for the WIN 35.065-2 analogs

were correlated with the structural features using Comparative Molecular Field Analysis (CoMFA) (Carroll *et al.,* 1991a). The CoMFA model developed was used in predicting new structures to be investigated. For example, the 3',4'-dichloro (RTI-111) and 3'-methyl-4'-chloro analogs (RTI-112) were predicted to be potent analogs and were found to be 29- and 28-times more potent than WIN 35,065-2 (Carroll *et al.,* 1992).

We have shown that the presence of a 2ß-substituent contributed substantially to the binding affinity of cocaine analogues at the dopamine transporter (Lewin *et al.,* 1992). Replacement of the 2ß-carbomethoxy group of cocaine by a hydrogen to give ß-tropacocaine, or epimerization to give pseudococaine. results in a 50- to 200-fold loss in potency. Replacement of the 2ß-carbomethoxy group with a 2ß-hydroxymethyl group to give (R)-3ß-(benzoyloxy)-2ß-hydroxymethyl-8-methyl-8-azabicyclo[3.2.l]octane resulted in a 5.6-fold loss in potency which could be partially restored (2.7) by acetylation to give 2ß-acetoxymethyl analog. Based on these results, we suggested a pharmacophore model in which the 2ß substituent enhances affinity to the binding site by an electrostatic interaction, probably serving as a hydrogen bond acceptor. In agreement with this possibility, we had noted that replacement of the carbomethoxy methyl group in 3ß-(substituted phenyl)tropan-2ß-carboxylic acids methyl esters by an isopropyl and phenyl group had only small effects on the affinity at the DA transporter, but caused large increases in the affinity for the NE and 5-HT transporter suggesting a highly specific interaction at the 2ß-position (Carroll *et al.,* 1992). In addition, we showed that the 2B-(1,2,4-oxadiazole) bioisosteres of the above compounds have binding potencies similar to their parent esters and that 3B-(4'-chlorophenyl)-2B-[3'-(4"-methoxyphenyl)-1',2',4'-oxadiazole-5-yl] tropane (RTI-141) which possesses an electron donating methoxy group is more potent than analogues RTI-143 and RTI-144 which contain electron withdrawing 4"-chloro and 4"-bromo groups, respectively, also suggesting an electrostatic contribution in the region of the 2ß-substituent (Carroll *et al.,* 1993).

In an unpublished study of 3ß-(4'-chlorophenyl)tropan-2ß-carboxylic amides, we have found that tert-amides such as the dimethyl amide RTI-129 were more potent at the DA site than primary and secondary amides, RTI-118 and RTI-106. respectively. Similarly, the cocaine N,N-dimethyl tert-amide RTI-160 is more potent than the N-methyl secondary and prima cocaine amides, RTI-66 and RTI-128. respectively. 3ß-(4'-Chlorophenyl)tropan-2ß-pyrrolidine carboxamide (RTI- 147) is both potent and selective for the DA site and will be a useful biochemical probe for studying this site. We have also shown that affinity for the DA site is increased by adding an oxygen atom to RTI-129 and RTI-160 to give 3ß-(4'-chlorophenyl)tropan-2ß-(N-methyl-N-methoxy)carboxamide (RTI-183) and (R)-3ß-(benzoyloxy)tropan-2ß(N-methyl-N-methoxy)carboxamide (RTI- 192). respectively. suggesting again that electrostatic interaction is important to the interaction of 2ß-substituents with the DA site. It is clear from these studies (Carroll *et al.,* 1992; Lewin *et al.,* 1992) that 2ß-substituents possessing increased potential for electrostatic interaction can lead to increased affinity for the DA site. However, unpublished results from our laboratory as well as results reported by others (Kozikowski *et al.,* 1992) suggest that this may not be a necessary requirement for high affinity for the DA site. These apparent differences could be explained if different ligands are binding at different sites or at the same site with different types of interactions with the DA transporter protein. Alternately. the DA transporter protein could be changing to adapt to different ligand types. Regardless of the reason, the high affinity observed for cocaine analogues possessing grossly different 2ß substituents raises the possibility that some of the pharmacological properties of the two types of analogues may be different. Additional studies are underway to gain information that will help explain the relationship between structural features at the 2ß-position, their interaction with the DA transporter protein, and their *in vivo* properties.

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BEHAVIORAL, PHARMACOLOGIC AND NEUROBIOLOGIC VARIABLES IMPORTANT TO THE ANALYSIS OF DRUG SELF-ADMINISTRATION: IMPLICATIONS FOR THE DISCOVERY OF POTENTIAL PHARMACOTHERAPIES

INTERRELATIONSHIPS BETWEEN DRUG SELF-ADMINISTRATION AND DRUG DISCRIMINATION

N. A. Ator

Drug self-administration procedures are used to study the reinforcing functions of psychoactive drugs (*i.e.,* whether a particular drug will maintain rates of responding greater than the drug vehicle alone). Those drugs that are reliably self-administered by laboratory animals typically have been those abused by humans and/or that have a profile of subjective effects similar to abused drugs. In drug discrimination procedures, drugs are established as discriminative stimuli (*i.e.,* a subject is trained to make one response if a particular drug dose has been administered and to make a different response if it has not), and the range of other drugs that will occasion the same response as the training drug can be studied. Because investigations have tended to presume that the effect that makes it possible to train a drug discrimination with a psychoactive drug is equivalent to the subjective effect of that drug, there has been the tacit or explicit assumption that information about the discriminative stimulus effect of a drug will yield information about its reinforcing efficacy and, by extension, abuse liability. For certain classes of drugs, sufficient data exist to compare the ability of a wide range of related compounds to maintain self-administration with their ability to substitute for a highly abused training drug in a drug discrimination procedure. For example, barbiturates are drugs with established abuse liability that also maintain self-administration in non-human primates. A wide range of CNS depressants that readily substitute for pentobarbital in drug discrimination studies with monkeys do also maintain self-administration. However, exceptions to this relationship emphasize that this is merely correlational and that similarities in discriminative stimulus effects of two drugs under one procedure may not be a function of the same variables that determine reinforcing efficacy.

Recently studies in baboons have investigated directly relationships between the discriminative and reinforcing effects of drugs. In one study, there was a disjunction between generalization to a particular dose of a training drug (midazolam) and self-administration of that dose by the same baboon in experimental sessions immediately following the drug discrimination. Furthermore, a drug (pentobarbital) that did not occasion midazolam-appropriate responding in drug discrimination, did maintain self-administration at a level comparable to that maintained by midazolam. These results point out the limitations of extrapolating drug reinforcement information from drug discrimination results and emphasize the role of the training drug in determining whether or not closely related drugs will substitute.

Another important area of investigation is whether a history of self-administering a drug may increase the probability of detecting subtle effects of that (or related) drugs. Conversely, it is possible that having been explicitly trained to discriminate a drug may increase the probability of self-administering that (or related) drugs. A history of drug discrimination training with midazolam apparently increased the probability of midazolam self-administration in baboons. Furthermore, recent data showed that sensitivity to the discriminative-stimulus effects of midazolam could be modulated differentially by behavioral experience with self-administered and response-independent midazolam.

CONTEXTUAL DETERMINANTS OF DRUG SELF-ADMINISTRATION IN MONKEYS.

Jack Bergman

Self-administration procedures in laboratory animals have been useful in the preclinical assessment of abuse liability and in evaluating therapeutic strategies for drug dependence. Although it is widely recognized that both pharmacological and contextual factors contribute to the effects of drugs in self-administration procedures, drug abuse research in laboratory animals has been directed primarily at evaluating pharmacological modification of drug-maintained behavior. From a practical viewpoint, however, pharmacological interventions are likely to serve only as adjuncts in long-term programs to treat drug dependence.

The present remarks are intended to review some of the available information describing contextual determinants of drug self-administration in monkeys. The role of schedule of availability in studies of drug self-administration in monkeys and non-pharmacological reduction of drug-maintained behavior will be discussed. With regard to schedules of availability, data illustrate that different patterns and rates of self-administration can be maintained under different schedule conditions and that the slope and position of dose-effect functions may be qualitatively different when self-administered drugs are studied under different schedules.

With regard to the reduction of drug self-administration, several studies have evaluated the effects of suppressing drug-maintained behavior in monkeys by noxious stimuli such as response-contingent electric shock. These studies indicate that such procedures are most effective only under restricted conditions, raising questions of practical application. Studies in monkeys employing programmed options that may be incompatible with drug self-administration also have proven successful in reducing drug intake. Such strategies may have wider therapeutic application and deserve further investigation.

COCAINE AND HEROIN SELF-ADMINISTRATION: BEHAVIORAL AND PHARMACOLOGIC VARIABLES AND THEIR IMPLICATIONS FOR THE DISCOVERY OF POTENTIAL PHARMACOTHERAPIES

S. I. Dworkin

A discussion of the importance of considering behavioral and pharmacologic variables in interpreting data obtained from self-administration studies will be presented. Some of the factors that will be addressed include the drug (differences between cocaine and heroin), dose, drug access and behavioral specificity of observed effects. Additionally, data obtained from studies evaluating the effects of a novel tropane analogue 2ß-propanol-3ß-(4-toluyl)-tropane (PTT) will be used to illustrate the importance of these variables. PTT, which is 20 times mom potent than cocaine in displacing [^{125}] RTI-55 binding, was evaluated for its effects of cocaine self-administration. Doses of the compound (10-90 µg/inf) maintained responding when substituted acutely for cocaine (0.33 mg/inf in rats responding under a fixed-ration 10 schedule of reinforcement. Moreover, a moderate dose of PTT (30 µg/inf was also able to engender and maintain self-administration in naive rats. The IP administration of PTT (1 or 3 mg/kg) resulted in a significant attentuation of cocaine self-administration. However, only the larger dose suppressed responding maintained by either heroin or food. The results from these-studies sug
gest that several different behavioral procedures should be used in the assessment of potential pharmacotherapies.

BEHAVIORAL, PHARMACOLOGIC AND NEUROBIOLOGIC MEASURES OF COCAINE REINFORCEMENT AND 'CRAVING' USING A MULTIPLE SCHEDULE IN THE RAT

G. F. Koob

Operant measures of drug reinforcement have long been used to measure the reinforcing properties of drugs of abuse. In primates, multiple schedules have been useful in characterizing not only the acute reinforcing actions of drugs but also in measuring of the reinforcing properties of stimuli associated with drug reinforcement. In the present series of studies a multiple schedule has been employed in the rat that is sensitive to pharmacologic, neurobiologic and behavioral manipulations. Male Wistar rats were trained to lever press for food and subsequently implanted with intravenous jugular catheters and trained in daily 2-hour sessions on a fixed-ratio multiple schedule alternating every 30 min for food and intravenous cocaine, in that order. Pairing of a light or tone with food or cocaine produced reliable lever pressing for this discriminative stimulus in daily 5 min pre-sessions prior to the start of the multiple schedule and priming with either cocaine or food selectively increased responding for the appropriate discriminative stimulus. Responding could be reinstated after extinction by presentation of the cocaine paired cue. Systemic administration of dopamine D-1 antagonists at low doses selectively decreased cocaine self-administration, and neurotoxin selective lesions of nucleus accumbens dopamine terminals also reduced responding for cocaine but failed to alter food responding. These results suggest that a food/drug multiple schedule in the rat may prove to be a valuable method for evaluatiing the neurobiological bases of the reinforcing actions of cocaine and the ability of cocaine to impart reinforcing properties to previously neutral stimuli ("craving").

EFFECTS OF POTENTIAL PHARMACOTHERAPIES ON BEHAVIOR MAINTAINED BY COCAINE OR BY ALFENTANIL

Gail Winger

Rhesus monkeys are given the opportunity to self-administer cocaine, or the opioid alfentanil: the drugs are delivered intravenously as a consequence of responses on an available lever in The animal's home cage. The schedule of drug delivery is FR 30 to 45". There are two daily sessions and each 130 min session consists of four components. Each component is as much as 25 min in duration and is followed by a 10 min time out period. The dose of drug (either cocaine or alfentanil) available during each component is different, and is controlled by the duration of the remotely-operated infusion pump: Thus, a dose-response function is obtained in each session, and pretreatment-induced changes in ED50 can be observed during the course of a single session.

Two strategies are used in searching for drugs that have potential use in the treatment of drug abuse: One is to identify an antagonist of the self-administered drug; the other is to find an agonist that suppresses self-administration of the drug. A sufficiently large dose of any drug will suppress drug-taking behavior, so an additional criterion for the second strategy is that the agonist suppress self-administration of one drug, either cocaine or alfentanil, in smaller doses than those required to suppress self-administration of the other drug. Thus, we are searching for an agonist-induced, drug-selective suppression of drug self-administration.

In the opioid system, drugs have been identified for both strategies. Thus, the opioid system can give us examples of what we are looking for in the more recalcitrant cocaine system. Quadazocine, the mu-selective opioid antagonist, when administered prior to a session in which various doses of alfentanil are available, produces a dose-dependent increase in the dose of alfentanil necessary to maintain high rates of responding. We am aware that opioid agonists have not been well-accepted in the treatment of opioid abuse, but theoretically, they should be quite successful, if motivation is high and compliance with medication-taking can

be assured. There are long-acting, opioid irreversible antagonists available. One of these, clocinnamox, is also able to increase the ED50 for alfentanil.
The agonist strategy, where compliance with medication administration is more likely to occur, has been demonstrated most clearly in the opioid class with buprenorphine. In our paradigm, very small doses of buprenorphine suppress alfentanil self-administration. Much larger doses are required to modify cocaine self-administration; doses that suppress cocaine self-administration; do not modify food-maintained behavior in a separate paradigm. This suggests that buprenorphine might be effective in treatment of opioid abuse with little problem of side effects. The more widely used methadone is also able to modify alfentanil-maintained responding. at doses that do not modify cocaine-maintained responding. A drug that is thought to have some buprenorphine-like properties, in that it has agonist and antagonist effects, and the antagonist effects are long-lasting, is NIH 10420, It, like buprenorphine, acts as a reinforcer. But when given-prior to sessions of alfentanil availability, it acts like an antagonist, rather than like an agonist. This is a remarkably curious outcome.

When we concentrate on trying to modify cocaine-reinforced behavior in a selective fashion, there is considerably more difficulty. The drugs that seem most reasonable to evaluate as antagonists are drugs that act on the D1 and/or the D2 receptor systems. There is some tendency for these drugs to produce increases in cocaine's ED50 (*i.e.,* look like antagonists), but this appears to be due to some extent to the ability of cocaine to antagonize the rate-decreasing effects of these drugs. This is evident by the fact that smaller doses than those necessary to modify cocaine self-administration are necessary to modify alfentanil self-administration. Furthermore, doses of these drugs that produce-a suppression of drug self-administration also produce marked changes in the general behavior of the subjects. It is unlikely that these drugs will be satisfactory in a treatment regimen.

Other drugs with potential cocaine antagonist effects are the atypical neuroleptics, amperozide and clozapine. These. drugs have some interaction with the dopamine system, but have a great deal more interaction with the serotonin system. Specifically, they are thought to have a large component of 5-HT2 antagonist action The interaction of these drugs with cocaine is quite different from that of the classic D1 and D2 antagonists. Clozapine produced similar effects at similar doses in animals self-administering cocaine and animals self-administering alfentanil. In each of the three alfentanil subjects, the smallest dose of clozapine produced increases in the maximum rate of alfentanil self-administration. Amperozide produced a somewhat different profile of action. It is the only drug we have evaluated that suppress cocaine-maintained responding at smaller doses than those necessary to suppress alfentanil-maintained responding. The dose differential was not great, but sufficiently unusual to rate notice.

We have done a little work with potential agonist interactions with cocaine self-administration. In this work, we did not use alfentanil as a control, but compared the ability of cocaine and GBR 12909 to modify cocaine self-administration. GBR 12909 was found to have reinforcing effects that appeared to be somewhat less than those of cocaine in that lower rates of responding were maintained by GBR 12909. The two drugs were virtually identical in their remarkable small and short-lived ability to suppress cocaine self-administration. In our hands, GBR 12909 did not appear to have use in treatment of cocaine abuse.

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DEPENDENCE STUDIES OF NEW COMPOUNDS IN THE RHESUS MONKEY, RAT AND MOUSE (1993)

M.D. Aceto, E.R. Bowman, L.S. Harris and E.L. May

All compounds, except nor binaltorphimine.HCl (nor BNI), dynorphin (1-13). dynorphin (2-17), naltrexone.HCl, (-)-nicotine, tartrate Pro-Leu-Gly-Amide (MIF), (-)-quadazocine·HCl, and morphine·SO_4 were supplied by Dr. Arthur Jacobson, Laboratory of Medicinal Chemistry, NIDDK, NIH. The identities of all the compounds, except those indicated above, were. unknown to us when they were originally submitted. These studies were conducted under the auspices of the Drug Evaluation Committee of the College on Problems of Drug Dependence.

Dependence-Liability Studies in Rhesus Monkeys

Substitution-for-Morphine (SDS) Test. Male and female rhesus monkeys (M. mulatta) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., of morphine SO_4 every 6 h. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 h and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-h observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score $\pm$ SEM was calculated and the data illustrated in figure form.

Precipitated-Withdrawal (PPT-W) Test. This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 h after the last dose of morphine. These animals were not in withdrawal. Naloxone·HCl (0.05 mg/kg, s.c.) served as the positive control.

Primary-Physical-Dependence (PPD) Study. Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with antagonist or abruptly withdrawn from the drug.

Rat-Infusion Studies

The continuous-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. 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 h. Occasionally, when deemed necessary, as with cocaine, infusions were given *via* the right jugular vein.

Substitution-for-Morphine (SM) Test. The rats received morphine·SO_4 (50 mg/kg/24 h on the first day, 100 mg/kg/24 h on the second day, and 200 mg/kg/24 h 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 0.5 h at 6, 24, 48, 72 and/or 96 h after stopping the infusion of morphine.

Primary-Physical-Dependence (PPD) Study. The rats received test compound, as specified above, for 6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

Mouse-Antinociception Tests

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED50's were. calculated by using computerized probit analysis.

Tail-Flick (TF) and (TF vs M) Assays. The procedure and modifications were described (D'Amour and Smith, 1941 and Dewey et al., 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 s under control conditions. Mice were injected with drug or vehicle and tested 20 m later. In the assay for antagonism of the antinociceptive effect, the potential antagonists were administered 10 m before the agonist, and evaluation occured 20 m later.

Phenylquinone Abdominal-Stretching (PPQ) Assay. The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drugs and 10 m later received 2.0 mg/kg ip of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten minutes after the PPQ injection, the total number of stretches per group were counted over a l-m period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response. was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay. The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 s or more, but no more than 30 s beyond the control time. Table 2 contains a summary of all the new data generated this year on compounds that had not previously been tested.

Calculation of Apparent pA_2 Using the tail-flick assay, the apparent pA_2 and 95% confidence limits were calculated using Schild and constrained plots as described in Tallarida and Murray (Manual of Pharmacologic Calculations with Computer Programs, 2nd ed., Springer Verlag, N.Y., 1987).

Briefly, mice were pretreated with vehicle or various doses of antagonist followed 10 min later by an injection of agonist. The mice were tested 30 min after receiving the antagonist. Dose-response lines for antinociception were plotted using at least 4 doses of each opioid agonist in the presence of vehicle or one of the selected doses of antagonist. ED50s were estimated according to the method of Litchfield and Wilcoxon (J. Pharmacol. Exp. Ther., 96, 399, 1949). Each dose ratio (x) was calculated by dividing the ED50 of the opioid in the presence of a given dose of antagonist by that of the agonist alone. Log (x-1) was plotted against the negative logarithm of the molar dose of the antagonist. At least 3 logs (x-1) were plotted. The pA_2 values for the antagonist were calculated from the point of intersection of the regression line with the abscissa.

Table 1

Comparitive DAtea(ED50, mg/kg s.c.) [95% C.L.] of selected standards in 4 Mouse Agonist-Antagonists Tests

Drug	Tail Flick	Tail Flick Antagonist	Phenylquinone	Hot-Plate
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·HCI	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)	- - - -
Naloxone·HCI	None at10.0	0.04 (0.01-0.09)	No Activity	- - - -
Naltrexone·HCI	None at 10.0	0.007 (.002-0.02)	No Activity	- - - -
Morphine·SO[b]	0.7[b] (0.4-1.5)	Inactive	0.4[b] (0.2-0.8)	3.1[b] (1.5-6.4)
Codeine·PO_4	- - -	Inactive	- - - (0.39-16.8)	6.4 (0.39-16.8)
Meperidine·HCI	- - -	Inactive	- - -	4.6 (1.8-11.7)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time

[b]ICR-Harlan-Sprague-Dawley Inc.

Table 2

Summary of New Hot-Plate Data on Previously Untested Compounds

NIH Compound	NAME	ED50 or % Activity (mg/kg)*
10602	- - -	0.5 (0.2 - 1.5)
10604	- - -	38% at 10 and 30
	- - -	Inactive
10605	- - -	Inactive
10606	- - -	Inactive
10607	- - -	Inactive
10612	- - -	Inactive
10613	Caffeine	Inactive
10614	Boldine	Inactive
10616	Flumazenil	Inactive
10617	(+)-Etorphine	Inactive
10618	- - -	Inactive
10619	- - -	Inactive
10620	(+)-Thevionone	Inactive
10621	(+)-Propylthevinol oxalate	Inactive
10623	- - -	13% at 1.0; 30% at
10626	(+)-N-n-Hexyl-normetazocine	Inactive
10627	(-)-N-n-Hexyl-normetazocine	3.4 (1.7 - 6.8)
10629	- - -	13% at 30.0
10630	- - -	3.7 (1.6 - 9.1)
10631	(-)-Thevinone	Inactive
10633	(+Decarbannoylphysovenine	Inactive
10634	- - -	0.3 (0.1 - 0.8)
10635	- - -	1.3 (0.6 - 3.2)
10636	- - -	1.2 (0.6 - 2.5)
10642	(-)-Bromoeseroline	Inactive
10644	- - -	0.3 (0.1 - 0.7)
10647	- - -	Inactive
10648	- - -	Inactive
10649	(-)-N-Benzylnometazocine	Inactive

Table 2 Summary of Hot-Plate Data (continued)

NIH Compound	NAME	ED50 or % Activity (mg/kg)*
10650	- - -	Inactive
10651	- - -	13% at 30.0
10652	NorLAAM	0.4 (0.2 - 1.0)
10653	(-)-α-N-Acetyl-N-N-dinormethadol	Inactive
10654	(-)-α-N-Acetyl-N-N-normethadol	Inactive
10655	(-)-α-N-Acetyl-N-N-dinormethadol	Inactive
10656	Naloxone benzoylhydrazone (BOZO)	Inactive
10657	- - -	Inactive
10658	- - -	6.9 (2.5 - 19.6)
10659	- - -	Inactive
10660	- - -	Inactive
10661	- - -	Inactive
10662	- - -	0.8 (0.3 - 2.3)
10663	Nalmefene-3ß-D-glucuronide	Inactive
10665	Etonitazene	0.003 (0.001 - 0.009)
10666	α-(±)-N-Normetazocine	25% at 10.0
10667(7410)	α-(±)-Metazocine	1.5 (0.8 - 2.9)
10670	- - -	13% at 30.0
10672	- - -	Tested
10673	α-(±)-N-Heptyl-N-Normetazocine	1.1 (0.4 - 3.4)
10674	- - -	11.5 (4.4 - 30.2)
10675	α-(-)-N-Heptyl-N-Normetazocine	2.4 (1.1 - 5.1)
10677	- - -	13% at 30.0
10678	- - -	11.5 (4.4 - 30.2)
10679	LAAM	6.7 (3.5 - 12.8)
10680	- - -	Inactive
10681	- - -	25% at 30.0
10682	Benzoylecgonine	25% at 30.0
10683	- - -	0.3 (0.1 - 0.9)
10684	- - -	13% at 30.0

*Inactive = Inactive at 1, 10 and 30 mg/kg

SUMMARY OF NEW DATA

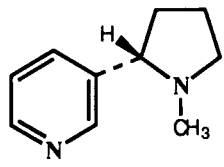
Compound NIH	Common Name	Chemical Name or Generic Class	MOUSE TF	MOUSE TF vs M	MOUSE PPQ	MOUSE HP	RAT SM/PPD	MONKEY SDS	MONKEY PPt-W PPD
9733	(-)-Nicotine-di-/-tartrate	Pyridyl-3-pyrrolidine	+[a,b]						
9752	(-)-Quadazocine	6,7-Benzomorphan	+[b,c]						
9930	Naltrexone	14-Hydroxydihydromorphone	+[b]						
10303	Dynorphin (1-13)	Peptide							Special[d]
10588	nor-Binaltorphamine	Bis-naltrexone	+[c]						
10661	Morphine-6-glucuronide	Morphine-6-glucuronide	+	+	+	+		+	
10662	Codeine-6-glucuronide	Codeine-6-glucuronide	+	+	+	+		+	
10665	Etonitazene	Benzimidazole	+[b,e]	+	+[b,e]	+		+	
10678		4-Phenylbutenylamine	+	+	+	+		+	+
10688		Piperidine-4-methanol							+
10697		(-)-6,7-Benzomorphan						+	
10698		(+)-6,7-Benzomorphan						+	
10707		3-Aminopiperidine	+	+	+	+		+	
10711		3,7-Diazobicyclononanone							
10714		3,7-Diazobicyclononanone							
10715		3,7-Diazobicyclononanone							
10716		3,7-Diazobicyclononanone	+	+	+	+		+[f]	
10718		1-Phenethylpiperidine-4-amide	+[e,g]	+	+	+		+	
10720		1-Phenethylpiperidine-4-amide	+[h]	+	+	+		+	
10735		(+)-3-Hydroxymorphinan							
10740		1-Phenethylpiperidine-4-amide	+	+	+	+		+	
10741		1-Phenethylpiperidine-4-amide		+	+	+		+	
10742		l-Phenethylpiperidine-4-amide	+[h]	+	+	+		+	
10743		1-Phenethylpiperidine-4-amide	+[h]	+	+	+		+	
10744		1-Phenethylpiperidine-4-amide	+	+	+	+		+	
10745		1-Phenethylpiperidine-4-amide	+[h]	+	+	+		+	
10748		(+)-3-Hydroxymorphinan	+	+	+	+		+	
10750		(+)-6,7-Benzomorphan	+	+	+	+		+	
10751		(+)-6.7-Benzomorphan	+	+	+	+		+	
10752		(+)-6,7-Benzomorphan	+	+	+	+		+	

SUMMARY OF NEW DATA (cont)

Compound NIH	Compount Name	Chemical Name or Generic Class	MOUSE TF	TF vs M	PPO	HP	RAT SM/PPD	SDS	MONKEY PPt-W	PPD
10753	Morphine Sulfate	Morphine	+	+	+	+		+		
10754		1-Phenylpiperidine-4-amide	+	+	+	+		+		
10755		1-Phenylpiperidine-4-amide	+	+	+	+		+		
10756		N-1-Hydroxyindanyl-piperidine	+[h]	+	+	+		+		
10757		N-Tetrahydronaphthylpiperidine-4-amide	+[h]	+	+	+		+		
10759	Ethylnarceine	Phenylethylamine	+	+	+	+		+		
10760	Narceine	Phenylethylamine	+	+	+	+		+		
10761		1-Phenethylpiperidine-4-amide	+[h]	+	+	+		+		
10762		1-Thienylethylpiperidine-4-amide	+[h]	+	+	+		+		
10763		1-Thienylethylpiperidine-4-amide	+[h]	+	+	+		+		
10765		1-Benzylpiperidine-4-amide	+	+	+	+		+		
10766		Piperidine	+	+	+	+		+		
10768		(-)-6,7-Benzomorphan	+	+	+	+		+		
10769		(+)-6,7-Benzomorphan	+	+	+	+		+		
10770		(-)-3-Hydroxymorphinan	+	+	+	+				
10771		(-)-3-Hydroxymorphinan	+	+	+	+				
10772		(+)-6,7-Benzomorphan	+	+	+	+		+		
10778		5-Phenylmorphan	+	+	+	+				
10779		5-Phenylmorphan	+	+	+	+		+		
10780		(-)-3-Hydroxymorphinan	+	+	+	+				
1078 1		(-)-3-Hydroxymorphinan	+	+	+	+				
10783		1-Phenethylpiperidine-4-amide	+[h]		+	+		+		
10799	Dynorphin (2- 17)	Peptide						+		
10800	MIF	Peptide					+[i]		+[i]	

[a]Special - Nicotine antagonism of morphine-induced antinociception. [b]Special - pA2 naloxone antagonism of nicotine antinociception. [c]special - pA_2 determination vs NIH 10672 and morphine. [d]Special - Behavioral effects in morphine-tolerant and morphine-non-tolerant rhesus monkeys (see table 1 of text) [e]special - Naloxone AD50 vs ED80 in TF and PPQ. [f]Preliminary - SDS in one monkey. Special - Naloxone AD50 vs ED80 of morphine in TF. [h]Special - Nlaoxone AD50 vs ED80 in T. [i]Special - MIF effects on tolerance to and dependence on morphine.

NIH 9733 (-), (S)-Nicotine di-1-tartrate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 5.2 (2.7 - 10.0)[a,b]
2) TF vs. M - 0.6 (0.03 - 15.0)[a,b]
3) PPQ - 1.3 (0.5 - 3.2)[a,b]
4) HP - 2.2 (1.6 - 3.0)[a,b]

[a] Reported previously (NIDA Monog. 34, 1981)
[b] doses expressed as salt

MONKEY DATA reported previously, op cit.

A. (SDS)

In the dose range of 0.03 - 0.75 (mg/kg, salt), nicotine did not substitute for morphine.

B. (PPt-W)

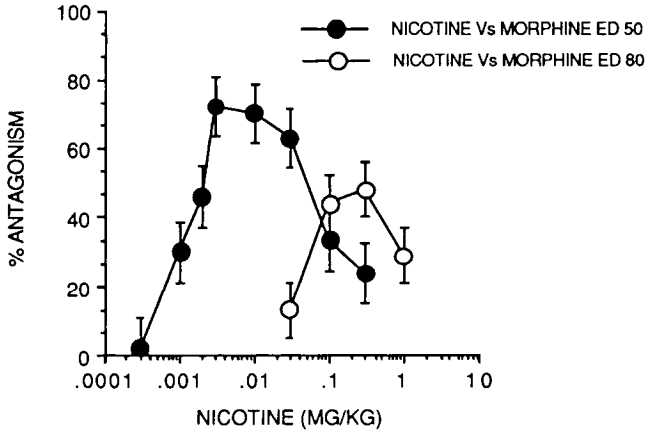
In the dose range of 0.06 - 0.96 (mg/kg, salt) some withdrawal signs noted, but data were confounded by unusual vehicle controls.

SPECIAL STUDY

The results of recent studies in humans suggested that nicotine not only reinforced smoking behavior because it released beta endorphin, but also negatively reinforced smoking behavior in response to nicotine withdrawal associated with low plasma concentrations. In our laboratory, we established over a decade ago that nicotine had opioid properties (op cit). Accordingly, we decided to characterize these properties further.

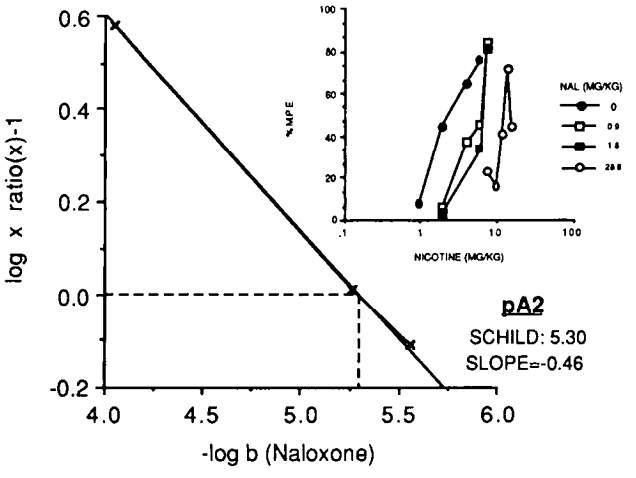
The results of the Nicotine-Morphine (NIC-MOR) antagonism studies are illustrated in the fig. Complex dose interactions involving both Nic and Mor were evident. Versus either the Mor MPE 50 or 80 similar biphasic responses were evident. The effect was much more robust when lower doses of Nic or Mor were involved. Nevertheless, Nic blocked the Mor MPE 50 in the remarkably low-dose range of 0.003 to 0.03 mg/kg. The descending portion of the biphasic curve probably reflects the emergence of Nic antinociception. These data did not suggest a competitive interaction rather, they indicated physiological antagonism.

NICOTINE VERSUS MORPHINE



The results of studies involving Naloxone-Nicotine (Nal-Nic) are shown in the fig. The data involving Nic alone and in combination with increasing doses of Nal are graphically displayed in the inset along with the Schild plot and slope function. The value of the slope function (-0.46) confirms that this interaction is not competitive. The apparent pA_2 was 5.3.

NALOXONE / NICOTINE APPARENT pA_2 (Schild)



NIH 9733 (-),(S)-Nicotine di-1-tartrate (cont.)

Summary

Nicotine produced antinociception in mice which was antagonized noncompetitively by naloxone. In addition, at significantly lower doses (2-3 orders of magnitude), nicotine antagonized morphine-induced antinociception. We tentatively propose that these opiatergic and anti-opiatergic properties may play a role in promoting and maintaining smoking behavior in man.

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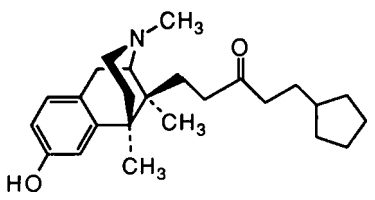
NIH 9752 (-)-Quadazocine, Win 44441-3



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF Inactive at 1.0, 10.0 and 30.0
2) TF vs M - 0.06 (0.01 - 0.39)
Time Course - 78% at 1 hr; 71% at 3 hr, 31% at 10% and 0% at 30 hr
3) PPQ - 44% at 0.001; 27% at 0.01; 33% at 0.1; 65% at 1.0 and 47% at 30.0
4) HP- No dose response
5) N - Inactive to 100.0

Reported previously. NIDA Res. Mong. 34, 1981.

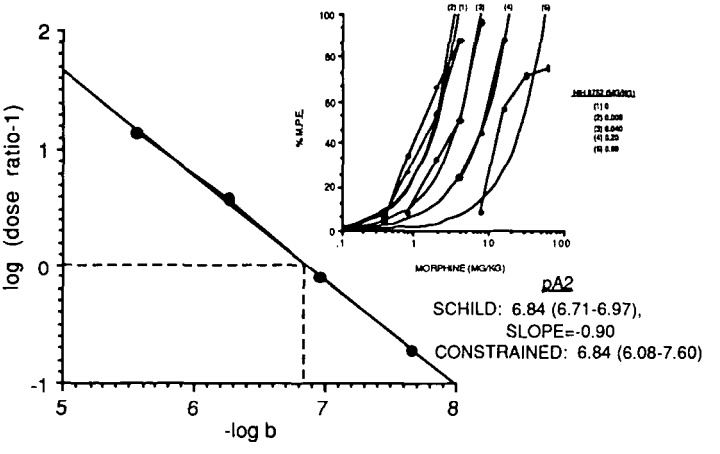
MONKEY DATA - Reported previously - op cit.

A. (SDS) Did not substitute for morphine, exacerbated withdrawal, and long duration of action.

B. (PPt-Withdrawal) Precipitated withdrawal and very long duration of action.

Apparent pA_2 Determination: NIH 9752/morphine and NIH 9752/NIH 10672 in TF test. Despite reports from this and other laboratories indicating that (-)-quadazocine was a potent mu antagonist of long duration (Aceto, et al, NIDA Res. Monog. 34, 1981, some investigators still describe this compound as a selective kappa antagonist. To resolve this issue, apparent pA_2s were calculated vs. morphine, a mu agonist and NIH 10672 or [5R-(5α,7α,8β)]-N-[7-(1-pyrrolidinyl)]-1-oxaspiro[4,5][dec-8-yl]-4-benzofuranacetamide·-HCl a purported potent and selective kappa agonist. For additional biological data on NIH 10672 consult (Aceto, et al., NIDA Res. Monog. 119, 1992).

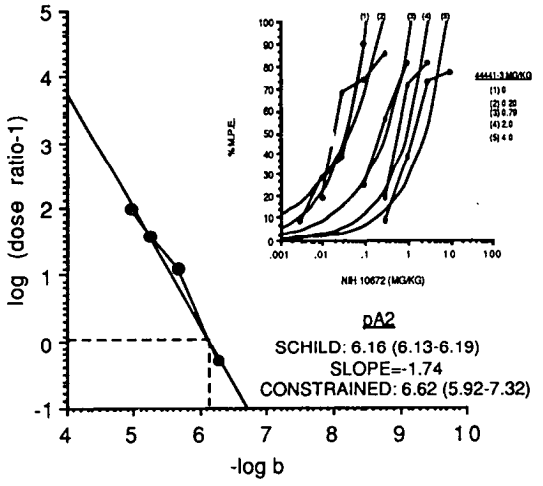
WIN 44441-3 (NIH 9752) VERSUS MORPHINE IN TAIL-FLICK
Apparent PA2-Schild (30 MIN)



As shown in the appropriate fig., an apparent pA_2 of 6.8 (6.71 - 6.97) and a slope of -0.9 were calculated for (-)-quadazocine versus morphine. Approximately the same values were calculated for naloxone vs morphine. The results indicate that (-)-quadazocine acts competitively on mu receptors. In sharp contrast, the apparent pA_2 of 6.16 (6.13 - 6.19) and slope of -1.7 for (-)-quadazocine vs NIH 10672 suggests that (-)-quadazocine has less affinity for kappa receptors than for mu and that the interaction is not competitive.

NIH 9752 (-)-Quadazocine, Win 44441-3 (cont.)

WIN 44441-3 (NIH 9752) VERSUS NIH 10672 IN TAIL-FLICK
Apparent pA2-Schild



Summary

(-)-Quadazocine is a competitive antagonist on mu receptors. It's action on kappa receptors is noncompetitive.

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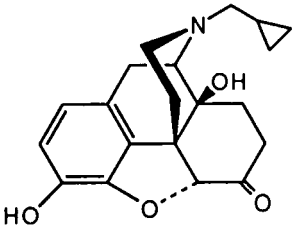
NIH 9930 Naltrexone·HCl



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0 and 30.0
2) TF vs M - 0.001 (0.0004 - 0.004)
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP - 10% at 20,40% at 50.0

Reported previously - NIDA Res. Monog. 43, 1983.

MONKEY DATA - Reported previously - op cit.

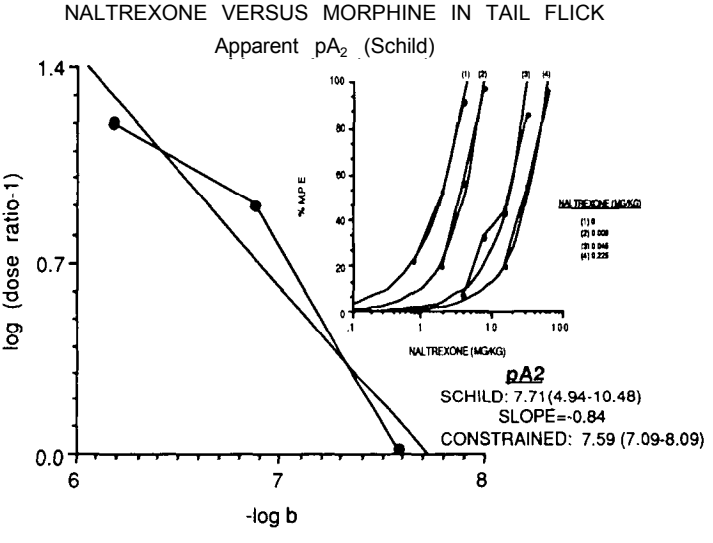
A. (SDS) Exacerbated withdrawal at 0.0625 mg/kg

NIH 9930 Naltrexone•HCl (cont.)

B . (PPt-W) Precipitated withdrawal. Potency approximately 10x naloxone

Apparent PA2 determination: naltrexone/morphine in TF test

Studies in this laboratory indicated that naltrexone was 10x as active as naloxone and approximately equipotent to nalmefene. The objective of the present study was to determine if the same relationships could be shown using pA_2s. As shown in the fig, naltrexone's pA2 is 7.71. The wide confidence limits reflect the fact that only 3 dose ratios were plotted. The pA2 calculated for naloxone was 7.2 and that for nalmefene was 8.0 (Aceto, et al, NIDA Res. Monog., 132, 1993). Thus, naltrexone's affinity for the mu receptor is approximately equal to that of nalmefene and is 5 x more than that of naloxone.



Summary

There is a rank-order correlation between pA_2s or affinity constants and potencies for naloxone, naltrexone and nalmefene. Naltrexone's affinity for the mu receptor is approximately equal to that of nalmefene.

NIH 10303 Dynorphin (1-13) (porcine) Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys

MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF[a]-
2) TF vs. M[a]-
3) PPQ[a]-
4) HP[a]-

[a]Not tested

MONKEY DATA
SDS

Special Study: Effects of Dynorphin (1-13) on Morphine-Tolerant and Non-Tolerant Monkeys

As shown in the accompanying table, morphine-dependent monkeys displayed fewer overt behavioral signs than monkeys that had not received morphine for varying periods when challenged with dynorphin (1-13). Dynorphin acted promptly and its overt actions had dissipated in approximately 30 m.

Comment:

Morphine-dependent monkeys appeared to be much less susceptible than non-tolerant animals to the effects of dynorphin (1-13).

Table 1. Scored Overt Behavioral Effects of Dynorphin (1-13) on Morphine-Tolerant and Non-Tolerant Rhesus Monkeys

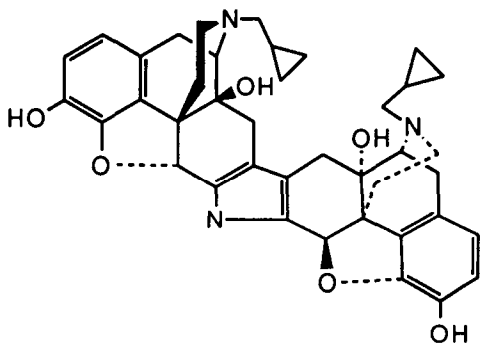
	Morphine-Dependent			Non-Tolerant					
Dose mg/kg i.v. in sterile saline	30			10				30	
Sex	M	M	F	F		F			
Monkey ID Number	1197	1233	1013	3010[b]	1083[c]	1195[a]	407[d]	1191[d]	1143[a]
Fell off Perch	–	–	–	–	–	–	–	–	√
Drowsiness	–	–	–	√	–	–	–	√	–
Slowing	–	√	–	–	–	√	√	–	–
Ataxia	–	–	√	–	–	√	√	√	√
Body Sag	–	–	–	√	√	√	√	√	√
Jaw Sag	–	–	√	√	√	–	√	√	√
Lying Down	–	–		√	–	√	√	√	√
Ptosis	√	–	√	√	√	–	√	√	–
Respiration Labored and/or Slow	–	–	√	√	√	√	–	√	√
Subdued or Tamed	–	–	–	√	–	–	–	√	–
Genital Touching	√	–	–	–	–	–	–	–	–
Restlessness	–	–	√	–	–	–	√	–	–
Retching	–	√	–	–	–	–	–	–	–
Vomiting	–	√	–	–	–	–	–	–	–
Piloerection	–	√	–	√	√	–	–	–	–
Irritability	√	–	–	–	–	–	–	–	–
Red Face	√	√	√	–	–	–	–	–	–
Scratching	–	–	–	–	–	–	–	√	–

aHad not received any drugs for at least a year; bHad not received morphine for at least 6 months; Wad not received morphine for at least 3 months; previously drug naive.

Monkeys 1143, 1195,1197, and 1233 received batch #030475, Peninsula Labs, Belmont, CA.

Monkeys 3010, 1083,1191, and 407 received batch #021697, Peninsula Labs, Belmont, CA.

NIH 10588, nor-Binaltorphimine dihydrochloride, nor-BNI



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change mg/kg)

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 - Inactive at 1.0, 10.0 and 30.0

Reported previously - NIDA Res. Monog. 105, 1991.

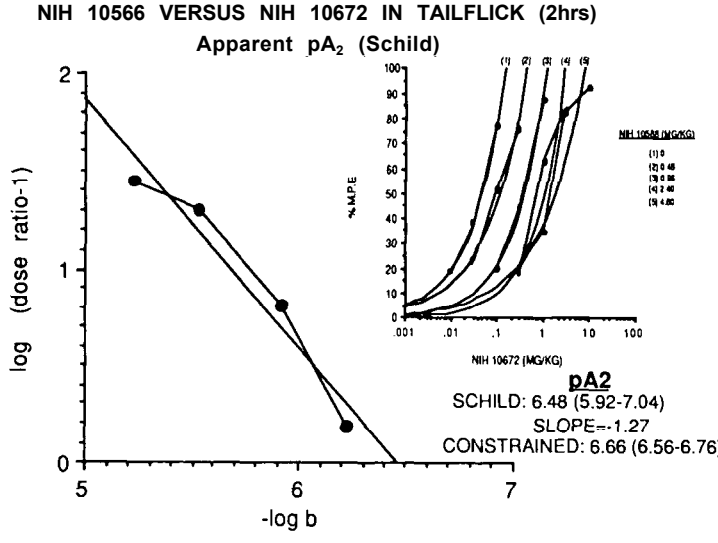
Special pA_2 determination in TF test

nor BNI is a highly selective kappa antagonist with a s.c. peak effect at 2 h (Takemori, et. al., JPET, 246, 1988). It was tested versus one of the most potent kappa agonists known (NIH 10672) or [5R-(5α, 7α, 8β]-N-[7-(1-pyrrolidinyl)]-1-oxaspiro[4,5][dec-8-y1]-4-benzofuracetamide·HCl. The apparent pA_2 and slope function (see fig.) indicates that the drug acts competitively with kappa agonists. Since the drug shows mu antagonist effects in the monkey, additional pA_2 studies involving morphine are indicated.

MONKEY DATA- (SDS) Reported previously - op cit.

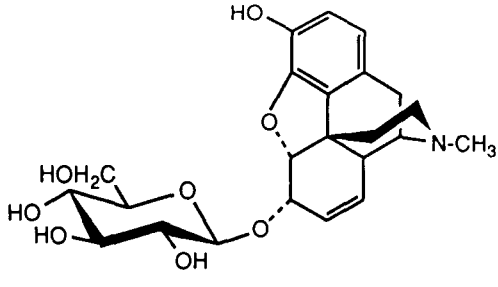
In the SDS test, nor -BNI exacerbated withdrawal at 2 and 8 mg/kg. The drug has mu-antagonist properties in rhesus monkeys.

NIH 10588, nor-Binaltorphimine dihydrochloride, nor-BNI (cont.)



- -

NIH 10661 6-O-ß-D-Glucopyranosylmorphine (Morphine-6-glucuronide)



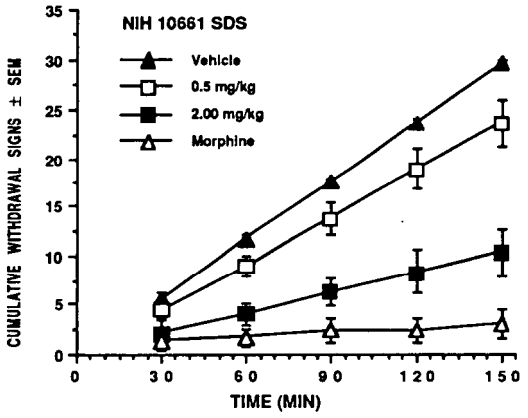
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - 0.7 (0.3 - 1.5)
2) TF vs. M. - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.2 - 0.6)
4) HP - 0.8 (0.3 - 2.3)

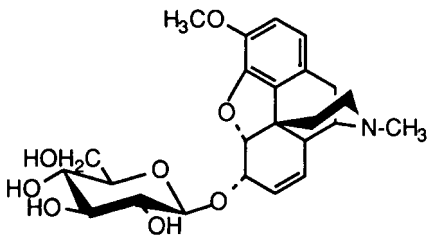
MONKEY DATA
S D S

Dose-dependent suppression of withdrawal signs was observed. Jaw sag and scratching were also noted at the high dose. In 2 monkeys receiving the highest dose, morphine was not required until 8 h had elapsed. Thus, the drug had a long duration of action and acted promptly. Potency is estimated as equivalent to that of morphine.

NIH 10661 6-O-B-D-Glucopyranosylmorphine (Morphine-6-glucuronide) (cont.)



NIH10662 6-O-ß-D-Glucopyranosylcodeine (Codeine-6-glucuronide)



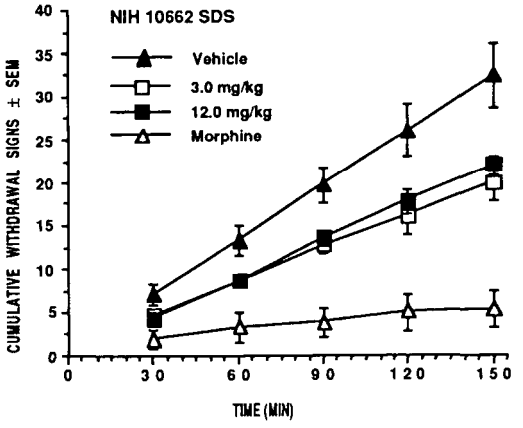
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 3.0 and 10.0, 16% at 30.0
2) TF vs. M - 11% at 1.0, Inactive at 10.0 and 30.0
3) PPQ-5% at 1.0, 11% at 10.0 and 23% at 30.0
4) HP - 0% at 1.0 and 10.0, 13% at 30.0

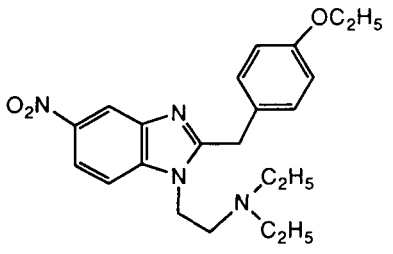
MONKEY DATA
(SDS)

NIH 10662 neither substituted completely for morphine nor exacerbated withdrawal in abruptly withdrawn monkeys. The partial suppression may be related to the reduced incidence of wet-dog shakes, coughing, vomiting, and retching and higher than usual vehicle scores.

NIH 10662 6-O-ß-D-Glucopyranosylcodeine (Codeine-6-glucuronide) (cont.)



NIH 10665 Etonitazene methanesulfonate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 0.005 (0.002-0.011)
2) TF vs. M-Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.0017 (0.0005-0.005)
4) HP - 0.003 (0.001 - 0.009)

Reported previously - NIDA Res. Monog. 119, 1992.

Special Test: Naloxone vs ED80 of NM 10665 in Tail-Flick and PPQ Assays

1) Naloxone AD50 = 0.3 (0.2 - 0.5) in Tail-Flick Test
2) Naloxone AD50 = 0.9 (0.4 - 2.5) in PPQ Test

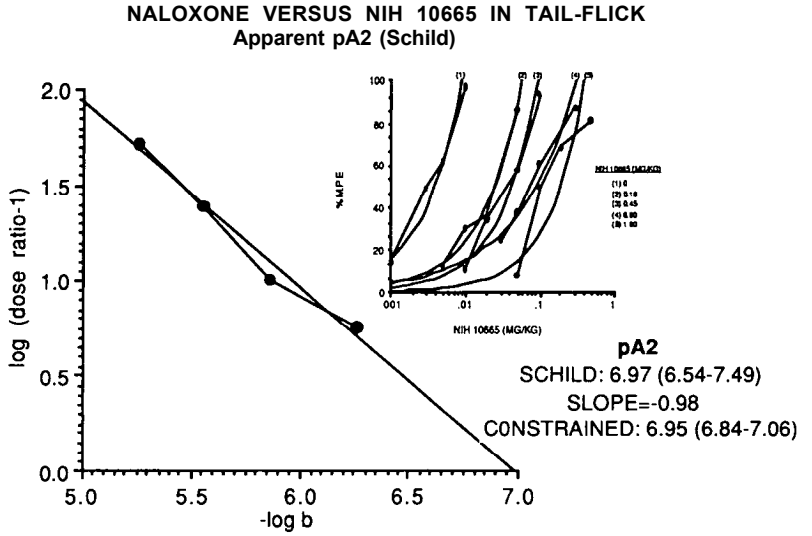
MONKEY DATA
(SDS)

See NIDA Res. Monog. 119, 1992.
NIH 10665 Etonitazene methanesulfonate (cont.)

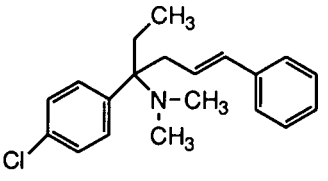
NIH 10665 Etonitazene methanesulfonate (cont.)

Substituted completely for morphine in abruptly withdrawn animals. Potency estimate 1500 x morphine.

Apparent pA2 Determination Because the AD50 vs morphine in the TF and PPQ tests suggested kappa properties, this test was conducted. The apparent pA2 calculated for NIH 10665 indicates that this drug is a mu agonist and that it interacts competivively with naloxone.



NIH 10678 (-)-1-(4)(Chlorophenyl)-N,N-dimethyl-1-ethyl-4-phenylbut-3-en-1-ylamine hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 13.7 (4.9 - 37.8)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.9 (0.2 - 3.6)[a]
4) HP - 11.5 (4.4 - 30.2)[a]

[a]Vechicle - 6% Tween 80 in water

NIH 10678 (-)-1-(4-Chlorophenyl)-N,N-dimethyl-1-ethyl-4-phenylbut-3-en-1-ylamine hydrochloride (cont.)

MONKEY DATA

A. (SDS)

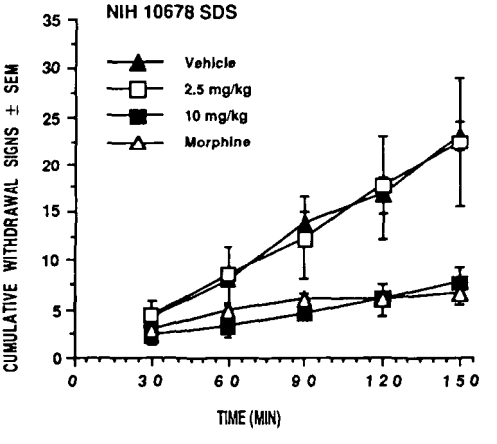
NIH 10678 substituted completely for morphine. Potency estimate was l/3 - l/2 that of morphine.

B. (PPD)

According to the observations noted below and summarized in the accompanying table, NIH 10678 probably has a mu-kappa opiate-dependence liability.

Overt signs typically associated with the administration of an opioid substance were seen after all injections of NM 10678 s.c. In addition, naloxone precipitated withdrawal in all the subjects receiving this compound. NIH 10678 produces physical dependence at pharmacologically active doses.

General Comment: NIH 10678 appears to be a typical mu agonist.



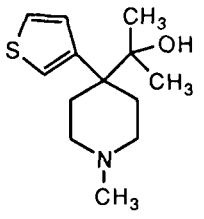
B. PPD) According to the observations noted below and summarized in the accompanying table, NIH 10678 probably has a mu-kappa opiate-dependence liability.

PRIMARY PHYSICAL DEPENDENCE STUDY WITH NIH 10678 IN RHESUS MONKEYS

DAY	DOSE (MG/KG)	COMMENTS
1 - 2	2.5[a]	Three male and 1 female rheus monkeys (Macuca mulatta) in the weight range of 3.6 - 4.1 kg at the start of the study served as subjects. The dose schedule is indicated below. Aqueous solutions (containing 1% Tween 80) of the test substance were given in a volume of 1/4 ml/kg S.C. The signs designated as body and jaw sag, chewing, eyelid ptosis, flushed face, restlessness, scratching and slowing were routinely scored during a 15 min period, 1/2 hr after the drug was given.
3	7.5[a]	
4 - 14	10.0	
15	10.0[a]	Precipitated Withdrawal (Day 15) All animals were injected with 0.25 mg/kg S.C. of naloxone.HC1. Withdrawal signs designated as vocalization, restlessness, coughing, lying on side or abdomen, vocalizes when abdomen palpated and rigid abdominal muscles were noted. Most of these signs lasted for approximately 45. min.

[a]Four injections daily at 12:00 Noon, 6:00 p.m., 12:00 Midnight and 6:00 a.m.
[b]One injection at 6:00 am.

NIH 10688 α,α,1-Trimethyl-4-(3-thienyl)-4-piperidinemethanol hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 5.4 (2.1 - 14.1)
4) HP - Inactive at 1.0, 10.0 and 30.0

Reported previously. NIDA Res. Monog. 132, 1993.

MONKEY DATA-Reported previously. NIDA Res. Monog. 132, 1993

A. (SDS) At 4, 16 and 24 mg/kg NIH 10688 neither substituted for morphine nor exacerbated withdrawal.

B. (PPD) New data.

According to the observations noted below and summarized in the accompanying table, NIH 10688 probably has a mu-kappa opiate-dependence liability.

Table. Primary Physical Dependence Study (PPD) with NM 10688 in Rhesus Monkeys

DAY	DOSE (mg/kg)	COMMENTS
	2	One female and 3 male rhesus monkeys (M. mulatta) (4/7 - 6.9 kg) served as subjects. Aqueous solutions
2-4	4	of test substances were administered S.C. in a volume of 0.25 ml/kg. Signs designated as ataxia, body sag,
5	8	eyelid ptosis, slowing, scratching and wide-eyed were scored during a 15-m observation period starting
6-8	12	0.5 h after drug was given. This observation schedule was continued throughout the 34-day study.
9-12	6	Dose was reduced to 6 mg/kg because of convulsions in 1 animal.
13	8	
14	10	
15	12	
16	12	Precipitated Withdrawal (Day 16)
		All animals were injected with 0.25 mg/kg S.C. of naloxone.HCl. Mu opiate withdrawal signs designated as lying on side or abdomen, fighting, avoids contact, vocalizes, crawling and rolling, restlessness, tremors, wet-dog shakes, yawning, retching, genital stimulation, vocalizes when abdomen palpated, and rigid abdominal muscles were noted. Other overt signs, not associated with mu-opiate withdrawal, designated as ataxia, chewing, tongue movements, dystonia, searching, stretching, rocking back and forth, aggression, piloerection and scratching were noted.
17-18	12	Same signs described after acute administration above.
19-21	14	
22-25	15	One monkey convulsed on day 23. Myoclonic convulsions, slowing, fell from perch.
26	7.5 & 15	Two monkeys received 7.5 mg/kg and two received 15 mg/kg.
27		Precipitated Withdrawal (Day 27)
		All animals received 0.25 mg/kg of naloxone. The following mu opioid withdrawal signs were. noted: lying on side, fighting, vocalization, restlessness, tremors, retching, vomiting, vocalizes when abdomen palpated, rigid abdominal muscles, yawning and genital stimulation. Other overt signs, not associated with mu-opioid withdrawal, designated as walking backwards, piloerection, and chewing were noted. One monkey had trouble finding its home cage. All monkeys seemed to move faster. All seemed to be aroused, all stopped scratching.

Table. Primary Physical Dependence Study (PPD) with NM 10688 in Rhesus Monkeys

DAY	DOSE (mg/kg)	COMMENTS
27-31 32-34	12 15	Abrupt Withdrawal (Day 34) All animals were abruptly withdrawn from drug and 16 h later opioid signs designated as yawning, lying on side or abdomen, fighting, avoids contact, vocalizes, restlessness, tremors, wet-dog shakes, retching, genital stimulation, vocalizes when abdomen palpated and rigid abdominal muscles were noted. Other overt signs, not associated with mu-opioid withdrawal were : body jerks, jaw movements, chewing, drowsiness, and scratching. The withdrawal syndrome peaked 15 hours after last injection and had completely abated 5 hr later. Comment: NIH 10688 definitely produces physical dependence in rhesus monkeys. However, although the withdrawal syndrome can be said to be associated with the mu opioid system, other systems are involved, possibly the kappa and/or dopaminergic system. Body weights remained relatively constant throughout the study.

NIH 10688 α,α,1-Trimethyl-4-(3-thienyl)-4-pipetidinemethanol hydrochloride (cont.)

RAT INFUSION - Reported previously. NIDA Res. Monog. 132, 1993.

A. (SM) Did not substitute for morphine but some suppression of behavioral withdrawal signs were apparent.

B. (PPD) Some evidence of mu opioid-like physical dependence.

Comment: This is an unusual compound. Although most of the results of the acute experiments are not remarkable, chronic studies in rats and monkeys indicate a mu and kappa opioid-like physical-dependence liability. The dopaminergic (D) system may also be involved.

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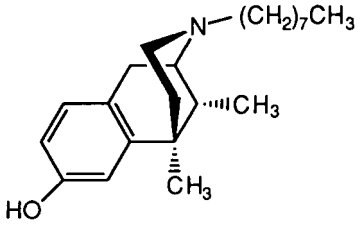
NM 10697 (-)-5-9α--Dimethyl-2'-hydroxy-2-*n*-octyl-6,7-benzomorphan hydrochloride



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF- 10.0 (4.3 - 23.2)[a,b]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a,b]
3) PPQ - 0.5 (0.2 - 1.5)[a,c]
4) HP - 5.4 (3.4 - 8.6)[a,d]

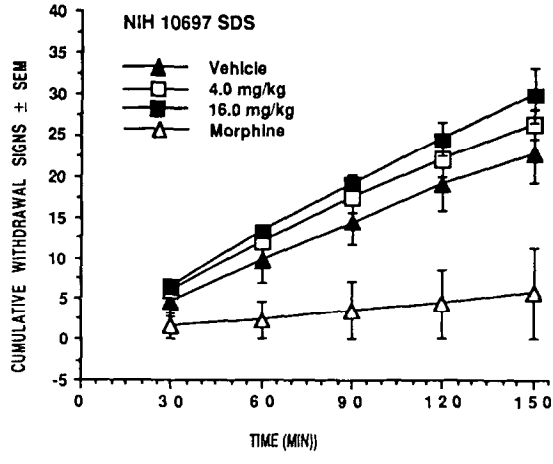
Vehicle - 50% DMSO in water
Vehicle - inactive
Vehicle - 47% inhibition
Vehicle - 25% inhibition

See previously reported NIDA REs. Monog. 132, 1993

MONKEY DATA
(SDS)

As illustrated in the accompanying graph, NIH 10697 did not substitute for morphine at doses of 4 and 16 mg/kg. The drug may have exacerbated withdrawal. All drugs used in the treatment regimens were dissolved in 25% aqueous hydroxypropyl-ß-cyclodextrin.

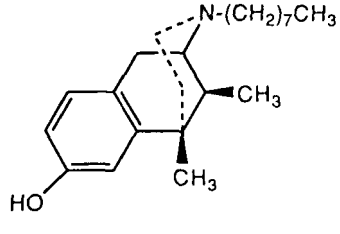
NM 10697 hydrochloride (-)-5-9α-Dimethyl-2'-hydroxy-2-*n*-octyl-6,7-benzomorphan (cont.)



Summary

Depending on the species, the drug shows opioid agonist or possible antagonist properties.

NIH 10698 hydrochloride (+)-5,9α-Dimethyl-2'-hydroxy-2- *n*-octyl-6,7-benzomorphan



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0 and 10.0,14% at 30.0[a,b]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0a
3) PPQ - 7.8 (1.2 - 49.5)[a,b]
4) HP - 11.1 (3.1 - 40.3)[a,c]

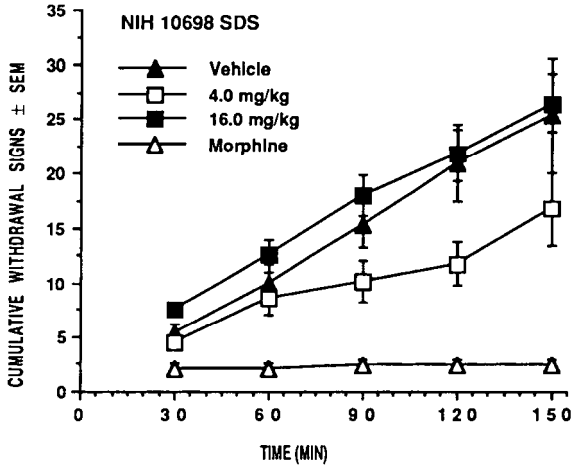
[a]Vehicle - 40% DMSO in water
[b]Vehicle - inactive
[c]Vehicle - 25% activity

Reported previously. See NIDA Res. Monog. 132, 1993.

NIH 10698 hydrochloride (+)-5,9α-Dimethyl-2'-hydroxy-2-*n*-octyl-6,7-benzomorphan (cont.)

MONKEY DATA
(SDS)

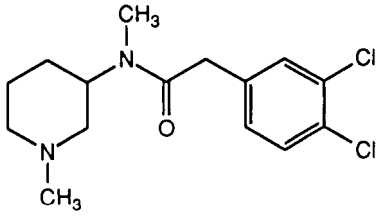
At doses of 4 and 16 mg/kg, NM 10698 neither substituted for morphine nor exacerbated withdrawal. The results are shown in the accompanying illustration. Vehicle consisted of 25% hydroxypropyl-ß-cyclodextrin in water.



Summary

Compared with the (-)-isomer (see NIH 10697), the (+)-isomer is less potent in the PPQ ane HP antinociceptive tests and is inactive in the monkey SDS.

NIH 10707 (±)-3-[N-Methyl-N-(3,4-dichlorophenylacetamido)]-1-methylpiperidine



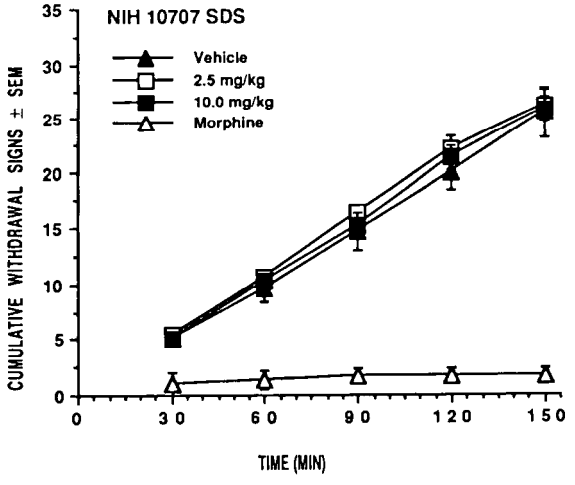
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 5.6 (1.6 - 19.6)[a]
4) HP - 25% at 1.0, 0% at 10.0 and 25% at 30.0[a]

[a]vehicle-H_3 PO_4 + water

MONKEY DATA
(SDS)

At doses of 2.5 and 10.0 mg/kg, no substitution for morphine or exacerbation of withdrawal was observed (see fig. NIH 10707 SDS). An increased incidence of retching was noted at the high dose. Vehicle was one drop of H_3PO_4 plus water.

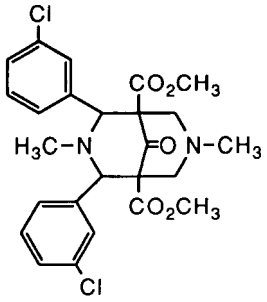


NIH 10707 (cont.) (±)-3-[N-Methyl-N-(3,4-dichlorophenylacetamido)]-1-methylpiperidine

SUMMARY

NIH 10707 displays antinociceptive activity in the PPQ test and questionable activity in the HP. In the dose range studied, no remarkable activity was observed in the monkey.

NIH 10711 4,6-Di-(3-chlorophenyl)-3,7-diazabicyclo[3.3.1]non-an-9-one 1,5-dicarboxylic acid dimethyl ester



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

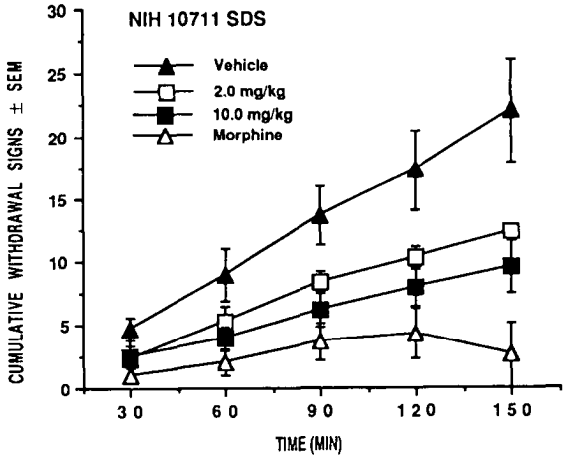
1) TF - Inactive at 1.0 and 10.0, 12% at 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 7% at 1.0, 31% at 10.0 and 10% at 30.0. Vehicle - 17% activity[a]
4) HP - Inactive at 1.0. 10.0 and 30.0[a]

[a]Vehicle - 0.5% gum tragacanth in H_2O. Drug not soluble in DMSO or dil HCl.

MONKEY DATA
SDS

As shown in the graph, NIH 10711 dose-dependently attenuated withdrawal but did not substitute completely for morphine. At the high dose, two important withdrawal signs, designated rigid abdomen and vocalization associated with palpation were suppressed beginning one h after drug was given. However, retching, wet-dog shakes and restlessness were not affected during the entire 2.5 h observation period.

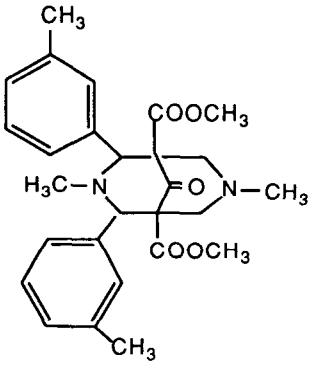
NIH 10711 4,6-Di-(3-chlorophenyl)-3,7-diazabicyclo[3.3.1]non-an-9-one 1,5-dicarboxylic acid dimethyl ester (cont.)



Summary

NIH 10711 attenuates withdrawal in monkeys but delayed effects or delayed absorption may preclude the full expression of activity Lack of antinociceptive activity in mice may involve similar pharmacokinetic considerations.

NIH 10714 3,7-Dimethyl-4,6-di-(3-methylphenyl)-3,7-diazabicyclo[3.3.l]nonan-9-one 1.5-dicarboxylic acid dimethyl ester

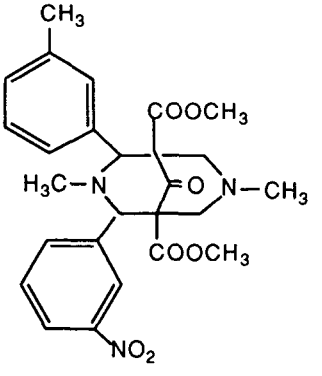


MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0, and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 30% at 1.0, 11% at 3.0, 41% at 10.0 and 46% at 30.0[a]
4) HP - 0% at 1.0, 13% at 10.0 and 30.0[a]

[a]Suspended in 5% gum tragacanth in water. Would not dissolve in dil acid or DMSO.

NIH 10715 3,7-Dimethyl-4,6-di-(3-nitrophenyl)-3,7-diazaibicyclo[3.3.l]nonan-9-one 1,5-dicarboxylic acid dimethyl ester



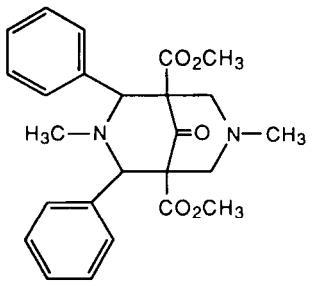
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0, 13% and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0 and 10.0, and 18% at 30.0[a]
4) HP -25% at 1.0, 13% at 10.0 and 25% at 30.0[a,b]

[a]Suspended in 5% gum tragacanth in water. Would not dissolve in dil acid or DMSO.

[b]Vehicle 13% activity.

NIH 10716 1,5-Dimethyl-4,6-diphenyl)-3,7-diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylic acid dimethyl ester



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - Inactive at 1.0, 10.0 and 30.0[a]
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle 0.5% gum tragacanth in [b]aqueous solution.

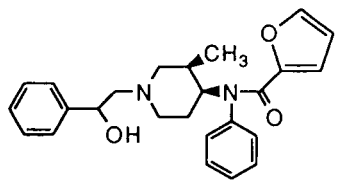
MONKEY DATA
(Preliminary SDS)

At 15-m intervals, doses of 0.8, 1.6, 3.2 and 6.4, respectively (cumulative dose of 12.0 mg/kg) of NIH 10716 neither attenuated, suppressed, nor exacerbated withdrawal. Vehicle was 30% Tween 80 in water.

Summary

This compound does not have significant biological activity in our tests. Solubility may be a factor.

NIH 10718 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenyl-2-furamide hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 7 x 10-4 (3.7 x 10" - 1.5 x 10-3)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a,b]
3) PPQ - 3.9 x 10^{-4} (1.6 x 10^{-4} - 9.4 x 10^{-4})[a]
4) HP - 4 x 10^{-4}-4 (1.7 x 10" - 1.0 x 10-3)[a,b]

[a]Vehicle 10% DMSO in water
[b]Straub tail starting at 0.01 mg/kg

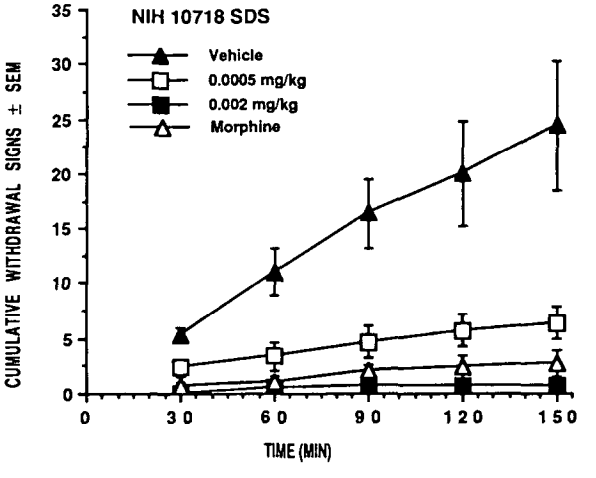
NIH 10718 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl-N-phenyl-2-furamide hydrochloride (cont.)

Special Test: Naloxone AD50 vs ED80 of NM 10718 in TF = 7.5 x 10^{-3} (2.0 x 10^{-3} 2.5 x 10^{-2})

Special Test: Naloxone AD50 vs ED80 of morphine TF - 0.007 (0.002 - 0.025)

MONKEY DATA
(SDS)

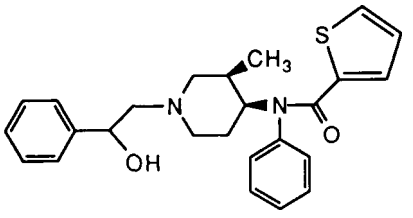
Complete substitution for morphine was observed with this drug. The onset of action was rapid and of at least of 2.5-h duration. Potency estimate is 3000 x that of the reference standard, morphine. At the high dose, the behavioral signs ataxia, body and jaw sag, slowing and drowsiness were observed. These signs are also seen when excessive does of morphine are used to terminate withdrawal in morphine-dependent monkeys.



Summary

Mu-opioid properties predominate in this compound.

NIH 10720 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl-N-phenyl-2-thiophenecarboxamide hydrochloride



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

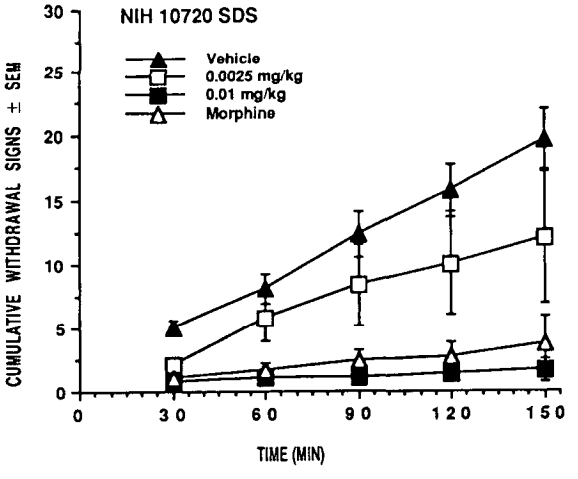
1) TF - 0.004 (0.002-0.01)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a,b]
3) PPQ - 0.003 (0.001 - 0.006)[a]
4) HP - 0.002 (0.0005 - 0.005)[a]

[a]Two drops of H3PO4 and water.
[b]One of 6 died at 30.0 mg/kg.

Special Test: Naloxone AD50 vs ED80 of NIH 10720 in TF = 0.007 (0.003 - 0.02)

MONKEY DATA
(SDS)

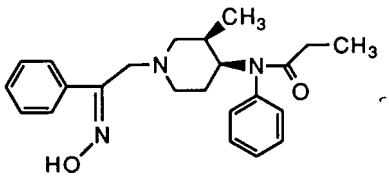
As shown in the accompanying figure, NIH 10720 dose-dependently substituted completely for morphine. In addition, at the high dose, the signs designated jaw sag, rubbing face, scratching and eyelid ptosis were seen. Onset of action was prompt. Offset was about 2.5 h. Potency estimate is 1000 x morphine.



Summary

The data suggest that NIH 10720 is a potent mu agonist.

NIH 10740 (±)-*cis*-N-[3-Methyl-1(2-iminohydroxy-2-phenylethyl)-4-piperidinyl]-N-phenylpropanamide (more polar isomer)



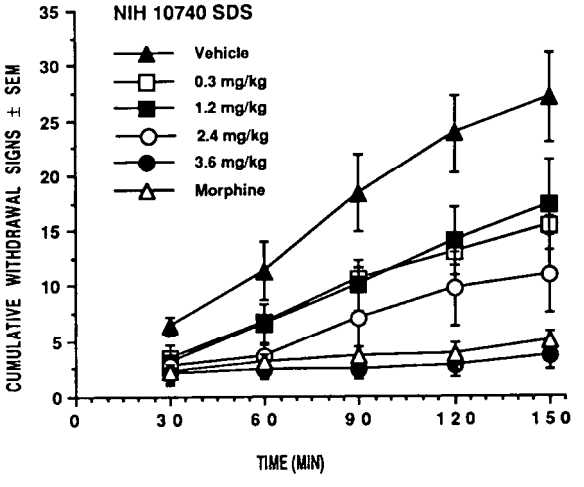
MOUSE DATA ED50 OR AD 50
(95% C.L.) or % change (mg/kg)

1) TF- 0.9 (0.3 - 2.4)[a]
2) TF vs. M Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.8 (0.2 - 2.6)[a,b]
4) HP - 1.8 (1.1 - 2.9)[a]

[a]Vehicle - 30% DMSO, H3PO4 and water
[b]Vehicle showed 20% activity in this test

MONKEY DATA
(SDS)

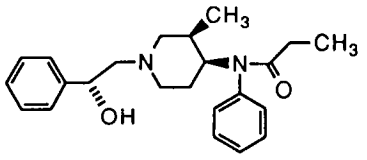
This compound dose-dependently substituted completely for morphine. The drug is estimated to be equipotent with morphine. Onset and offset of action are also similar. Vehicle was 20% Tween 80, phosphoric acid and water.



Summary

Strong mu-opioid properties are associated with this compound.

NIH 10741 (ßS,3R,4S)-(+)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-pipetidinyl[-N-phenylpropanamide hydrochloride or (BS,3R,4S)-(+)-*cis*-ß-Hydroxy-3-methylfentanyl hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF-2x10-4(1x10-4-3x10^{-4})
2) TF vs. M - Inactive at 1.0. 10.0

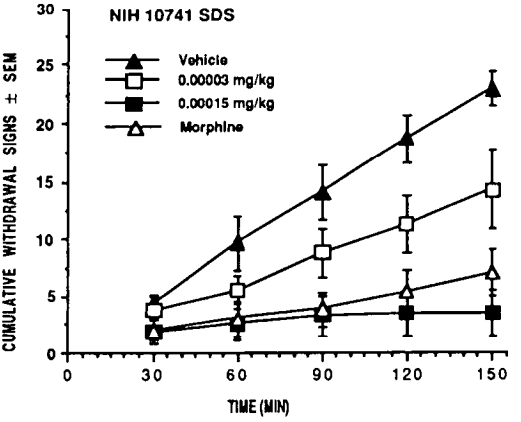
3) PPQ - 9 x 10-5 (3 x 10-5 - 2 x 10^{-4} and 30.0[a])
4) HP - 1 x 10-4 (5 x 10-5 - 2 x 10^{-4})

[a]One mouse found dead 1 hr after drug. All mice lost righting reflexes at 1.0 and 3.0. All mice were immobile but responded to touch at 1.0 and 3.0.

Special Test: Naloxone AD50 vs ED80 of NIH 10741 in TF = 8.3 **x** 10^{-3} (4 **x** 10^{-3} - 1.7 x 10^{-2})

MONKEY DATA
SDS

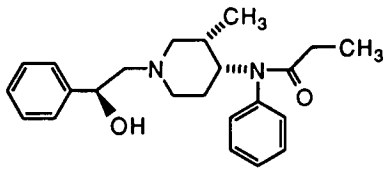
This compound substituted completely for morphine. Onset of action was rapid and offset of action was about 90 m. Peak effect estimated to occur at 60 m. NIH 10741 is 20,000 to 50,000 times more potent than morphine.



Summary

This is an extremely potent mu agonist.

NIH 10742 (ßR,3S,4R)-(-)- *cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl 4-piperidinyl]-N-phenylpropanamide hydrochloride or (ßR,3S,4R)-(+)-*cis*-ß-Hydroxy-3-methylfentanyl hydrochloride



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

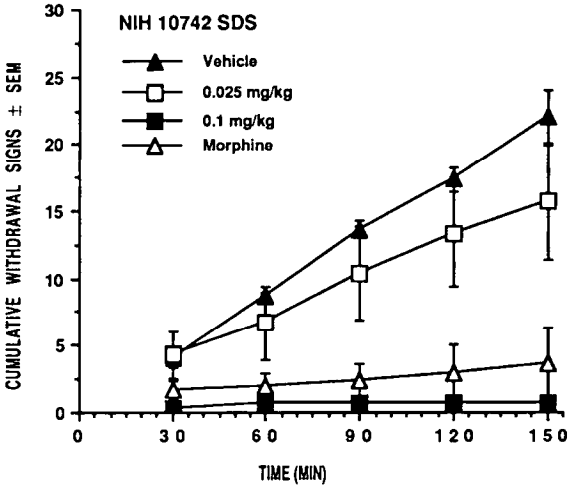
1) TF - 0.06 (0.03 - 0.11)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.03 (0.01 - 0.07)
4) HP - 0.08 (0.003 - 0.02)[a]

[a]Loss of righting reflex at 30.0
[b]Straub tail and increased locomotor activity beginning at 0.2 mg/kg

Special Test: Naloxone AD50 vs ED80 of NIH 10742 in TF = 0.03 (0.01 - 0.09)

MONKEY DATA
SDS

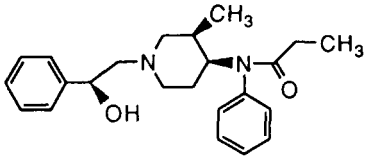
NIH 10742 produced a dose-related reduction in withdrawal signs (see fig.). In addition, the monkeys receiving the high dose did not require the usual injection of morphine at noon. This compound acted promptly, had a longer duration of action and is estimated to be 30 x more. potent than morphine.



NIH 10742 (ßR,3S,4R)-(-)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride or (BR,3S,4R)-(+)-*cis*-ß-Hydroxy-3-methylfentanyl hydrochloride (cont.)

Summary All the data indicated that NIH 10742 had opioid properties and that the mu component was well represented. The drug may have a longer duration of action compared with the reference compound morphine.

NM 10743 (BR,3R,4S)-(-)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl[-N-phenylpropanamide oxalate or (BR,3R,4S)-(-)-*cis*-ß-Hydroxy-3-methylfentanyl oxalate



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - 8.0 x 10^{-4} (3.0 x 10^{-4} - 2.0 x 10^{-3})
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.2 x 10^{-4} (2.1 x 10^{-5} - 6.5 x 10)
4) HP - 1.3 x 10^{-3} (4.0 x 10^{-4} - 4.6 x 10^{-3})[a]

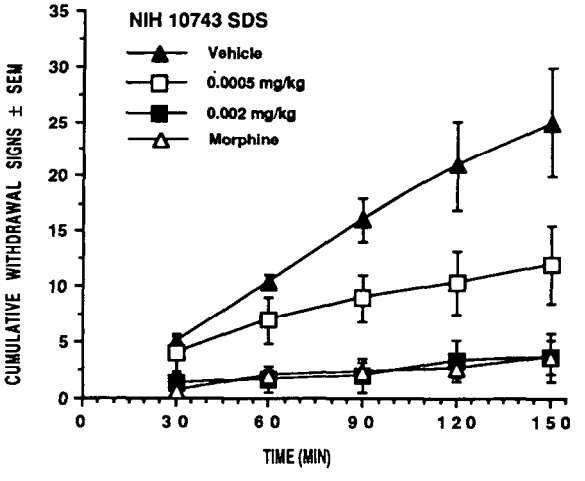
[a]Straub tail and increased locomotor activity beginning at 0.1 mg/kg.

Special Test: Naloxone AD50 vs ED80 of NIH 10743 in TF = 3.5 x 10^{-2} (1.0 x 10^{-2} - 1.1 x 10^{-1})

MONKEY DATA

As illustrated in the fig., NIH 10743 SDS, this compound substituted completely for morphine at 0.002 mg/kg. Furthermore, t he action was dose-related. NIH 10743 acted promptly; its duration of action was longer than that of morphine and the potency estimate is approximately 1500 x morphine.

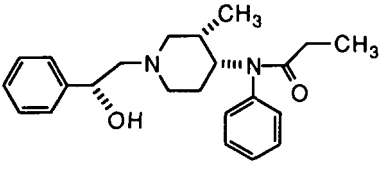
NIH 10743 (ßR,3R,4S)-(-)-cis-N-[1-(2(B)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl[-N-phenylpropanamide oxalate or (ßR,3R,4S)-(-)-*cis*-ß-Hydroxy-3-methylfentanyl oxalate (cont.)



Summary

NIH 10743 behaved as if it were a typical, very potent, mu opioid agonist.

NIH 10744 (ßS,3R,4S)-(+)- *cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl[-N-phenylpropanamide oxalate or ßS,3R,4S)-(+)-*cis*-ß-Hydroxy-3-methylfentanyl oxalate



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

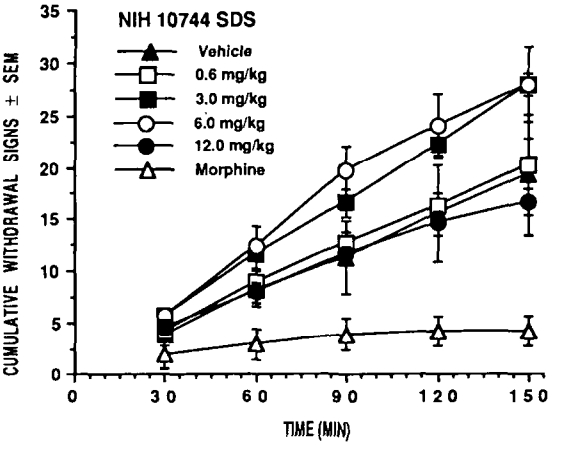
1) TF - 13.0 (5.6 - 30.6)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.6 (0.2 - 2.1)
4) HP - 2.1 (0.6 - 7.8)

NIH 10744 (ßS,3R,4S)-(+)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl[-N-phenylpropanamide oxalate or ßS,3R,4S)-(+)-*cis*-ß-Hydroxy-3-methylfentanyl oxalate (cont.)

MONKEY DATA

SDS

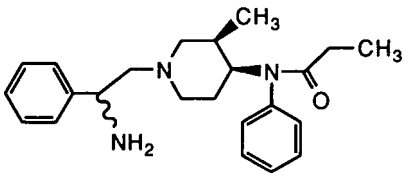
In the dose range of 0.6 - 12.0 mg/kg, NIH 10744 did not substitute for morphine. In fact, it appeared to exacerbate withdrawal in a dose-related manner at the three lower doses. However, at the highest dose, the drug's effects were like those of the vehicle. The data are illustrated in the drug's figure designated NIH 10744 SDS. In addition, salivation was noted at the highest dose in two monkeys.



Summary

NIH 10744 was active in the mouse antinociceptive assays. The activity could reflect mu and/or kappa opioid activity. However, in the monkey. the drug had a biphasic effect (behaved as a weak mu antagonist at the low dose but had no effect at the high dose).

NIH 10745 (±)-*cis*-N-[1-(2-Amino-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide dihydrochloride



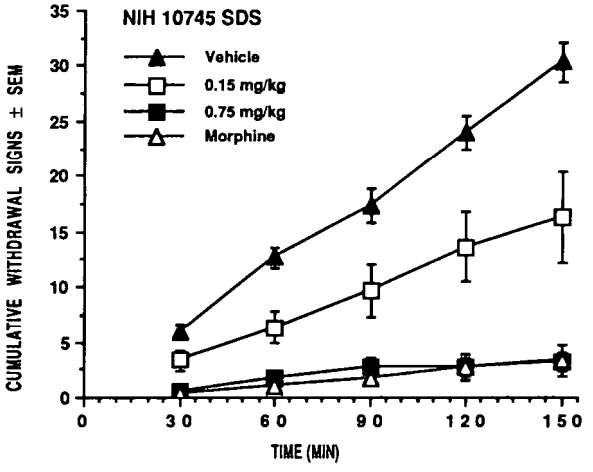
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - 0.6 (0.3 - 1.4)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ -0.09 (0.03 - 0.20)
4) HP - 0.7 (0.3 - 1.3)

Special Test: Naloxone AD50 vs ED80 of NIH 10745 in TF = 0.03 (0.008 - 0.1)

MONKEY DATA

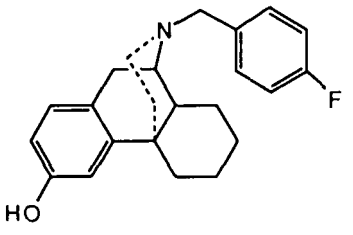
As shown in the accompanying illustration, NIH 10745 substituted completely for morphine. The action was prompt and dose-related. The drug appeared to be approximately 4-6 x more potent than morphine. Duration of action was similar to that of the reference control.



Summary

This compound showed a profile of activity like that of a mu opioid agonist.

NM 10748 (+)-N-(4-Fluorobenzyl)-3-hydroxymorphinan oxalate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 12.0 (5.9 - 33.7)[a]
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

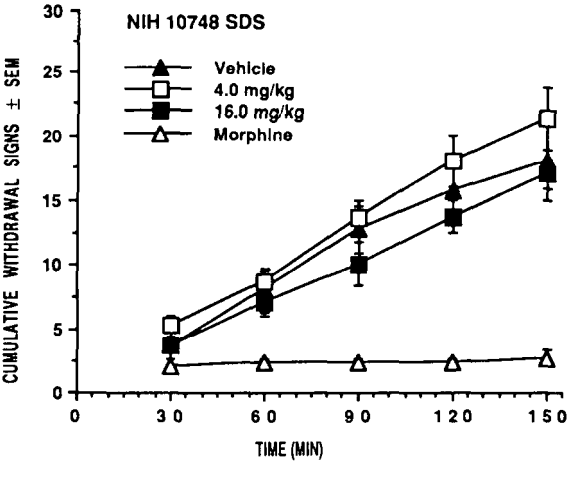
[a]Vehicle-5% Tween 80 in water

MONKEY DATA
(SDS)

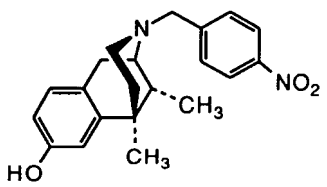
NIH 10748 neither substituted for morphine nor exacerbated withdrawal at doses of 4 and 16 mg/kg. One monkey at the high dose retched and vomited often. Vehicle was 25% hydroxypropyl-B-cyclodextrin in water.

Summary

Some weak activity in the PPQ test and possible weak mu antgonist properties are associated with NIH 10748.



NIH 10750 (-)-5,9α-Dimethyl-2'-hydroxy-2-(4-niuobenzyl)-6,7-benzomorphan oxalate



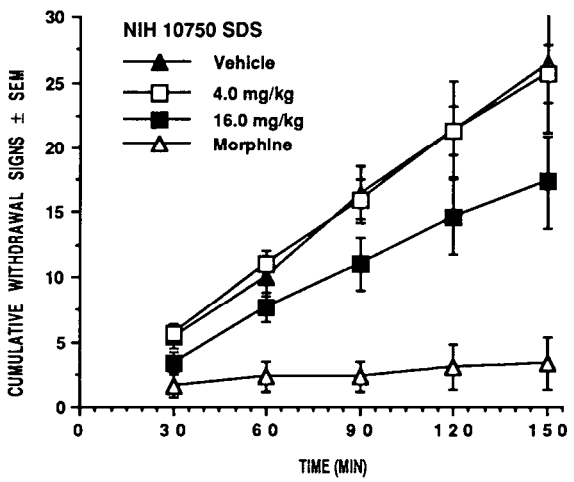
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 14% at 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 6% at 1.0, 13% at 10.0 and 63% at 30.0[a]
4) HP-0% at 1.0, 13% at 10.0 and 30.0[a]

[a]Vehicle - 5% Tween 80 aqueous suspension

MONKEY DATA
(SDS)

Analysis of variance (Kruskal-Wallis test) of the data at 120 m revealed significant differences among the treatment regimens (see fig NIH 10750-SDS). Post hoc comparisons between the individual treatment regimens and vehicle controls indicated that only the morphine control data were significantly different from those of vehicle controls. It was concluded that NIH 10750 neither suppressed nor exacerbated withdrawal. Vehicle was 25% hydroxypropyl-ß-Cyclodextrin in water.

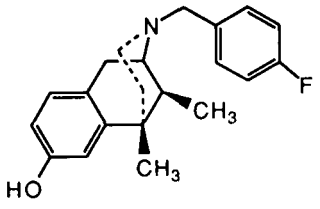


NIH 10750 (-)-5,9α-Dimethyl-2'-hydroxy-2-(4-nitrobenzyl)-6,7-benzomorphan oxalate (cont.)

Summary

Except for some weak antinociceptive activity in the PPQ test, NIH 10750's actions were not remarkable. The drug is virtually free of in vivo mu or kappa opioid activity.

NIH 10751 (+)-5,9α--Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan oxalate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

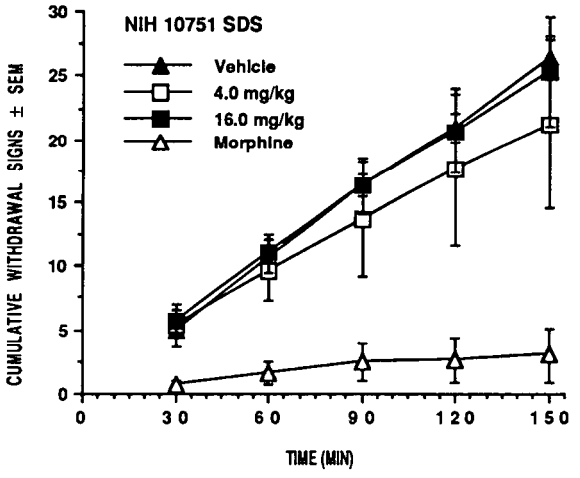
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 11% at 1.0, 8% at 10.0 and 14% at 30.0[a]
4) HP - Inactive at 1.0, and 10.0, 13% at 30.0

[a]Vehicle - 5% Tween 80 aqueous suspension

MONKEY DATA
(SDS)

As shown in the accompanying fig. (NIH 10751-SDS), this compound neither suppressed nor exacerbated withdrawal at doses of 4 and 16 mg/kg in maximally-morphine-dependent rhesus monkeys. Vehicle was 25% hydroxypropyl-ß-cyclodextrin in water.

NIH10751 (+)-5,9 α-Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan oxalate (cont.)

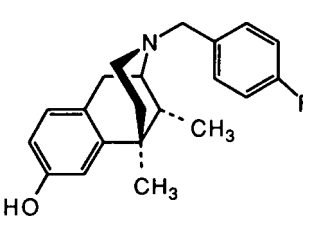


Summary

The results suggested that the compound had no apparent mu or kappa opioid activity or that solubility may be a problem.

- -

NIH 10752 (-)-5,9α-Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan oxalate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

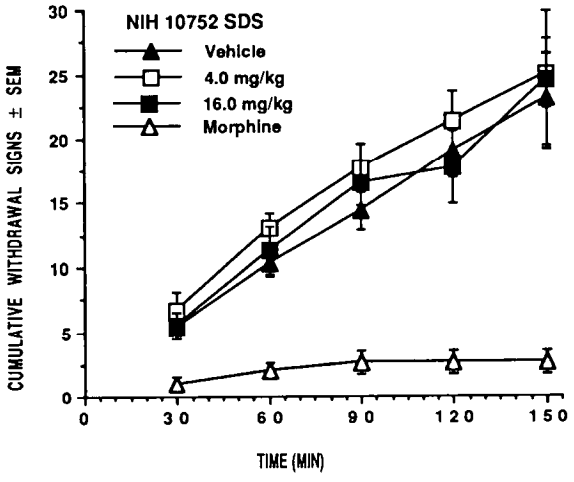
1) TF - Inactive at 1.0 and 10.0[a] and 15% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 6% at 1.0, 17% at 10.0 and 49% at 30.0[a]
4) HP - Inactive at 1.0, and 10.0, 15% at 30.0

Vehicle - 5% Tween 80 aqueous suspension

NIH 10752 (-)-5,9α-Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan oxalate (cont.)

MONKEY DATA
(SDS)

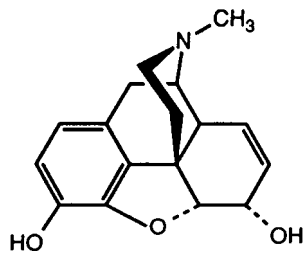
This compound neither exacerbated nor suppressed withdrawal at 4 and 16 mg/kg in abruptly withdrawn morphine-dependent monkeys (see fig. NIH 10752-SDS). Vehicle was 25% hydroxypropyl-ß-cyclodextrin in water.



Summary

The drug had some weak antinociceptive activity in the PPQ test. Lack of activity in the TF, TF vs M, HP tests and in morphine-dependent monkeys suggested that it had little, if any, mu or kappa opioid activity.

NIH 10753 Morphine sulfate pentahydrate

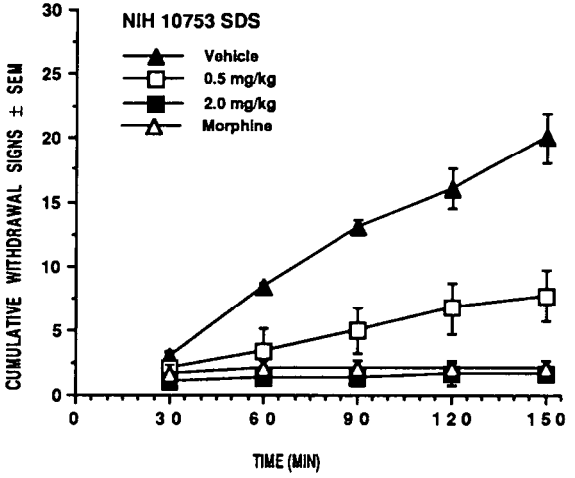


MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 0.7 (0.4 - 1.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.2 - 0.8)
4) HP - 3.1 (1.5 - 6.4)

MONKEY DATA
(SDS)

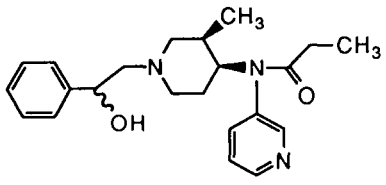
NM 10753 substituted completely and dose-dependently for morphine. The drug acted promptly and its duration of action was at least 2.5 h. The drug is about as potent as morphine.



Summary

This drug displays a profile of activity which suggests a strong mu-opioid component. It should be noted that NIH 10753 was submitted as an unknown and was tested “blind”.

NIH 10754 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidnyl]-N(3-pyridinyl)propanamide dihydrochloride

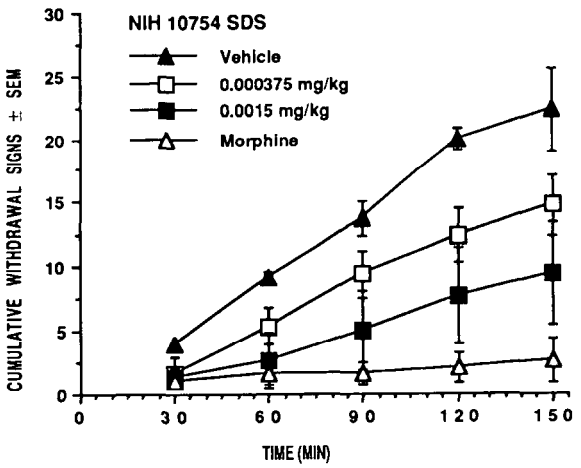


MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - 0.001 (0.0005 - 0.003)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 4.0 x 10-4 (2x 10-4 x 1 x 10^{-3})
4) HP - 0.004 (0.001 - 0.01)[b]

MONKEY DATA
SDS

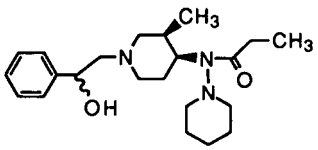
NM 10754 dose-dependently substituted for morphine. However, although onset of action was rapid, duration of action was about 90 m. Potency is estimated as 2000 x morphine.



Summary

NIH 10754 is a potent mu opioid agonist.

NIH 10755 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(1-piperidinyl)propanamide dihydrochloride



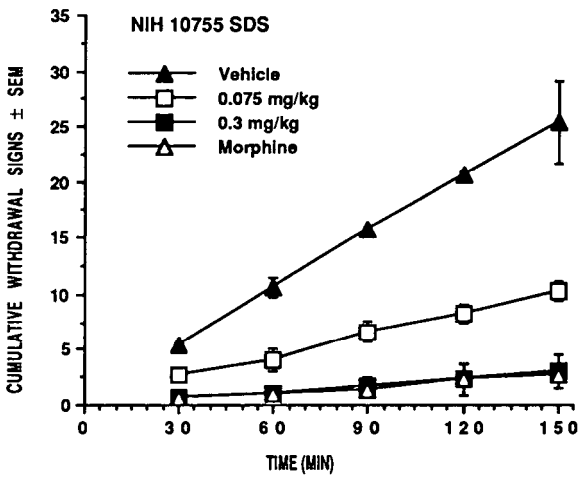
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 0.12 (0.07 - 0.20)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPO - 0.07 (0.02 - 0.21)
4) HP - 0.16 (0.08 - 0.33)

Special Test: Naloxone antagonism of NIH 10755 (ED80) in TF, AD50 = 0.04 (0.02 - 0.10).

MONKEY DATA
SDS

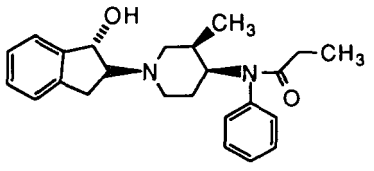
As shown in the accompanying graph, NIH 10755 dose dependently substituted completely for morphine. The drug is approximately 10 x more potent than the reference compound morphine Onset of action was prompt. Offset was at least as long as that of morphine or at least 2.5 h.



Summary

The data indicate that NIH 10755 is a potent mu opioid agonist.

10756 (±)-*cis*-N-[1-(2(*trans*-1-Hydroxy)indanyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



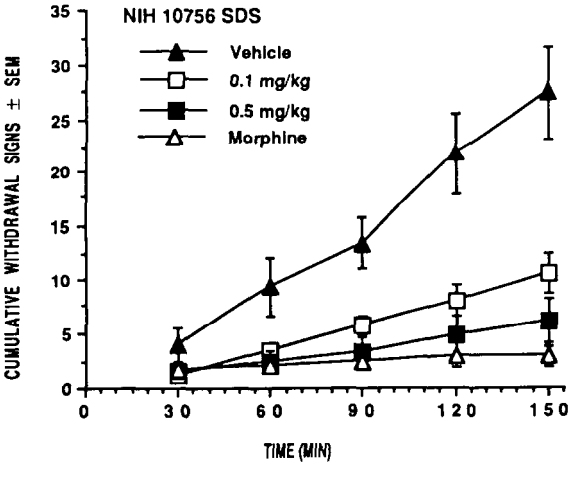
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 0.14 (0.06 - 0.32)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.03 (0.01 - 0.07)
4) HP - 0.18 (0.07 - 0.48)

Special Test: Naloxone antagonism of NIH 10756 (ED80) in TF, AD50 = 0.11 (0.05 - 0.26)

MONKEY DATA
SDS

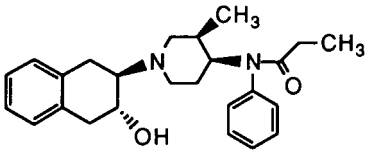
NIH 10756 substituted completely for morphine (see graph). The potency estimate was 6 x morphine. The drug acted promptly. However, its duration of action was less than that of morphine.



Summary

This profile of activity suggests that 10756 is a potent mu agonist.

NIH 10757 (±)-*cis*-N-[1-(2-(trans-3-Hydroxy-1,2,3,4-tetrahydro)naphthyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

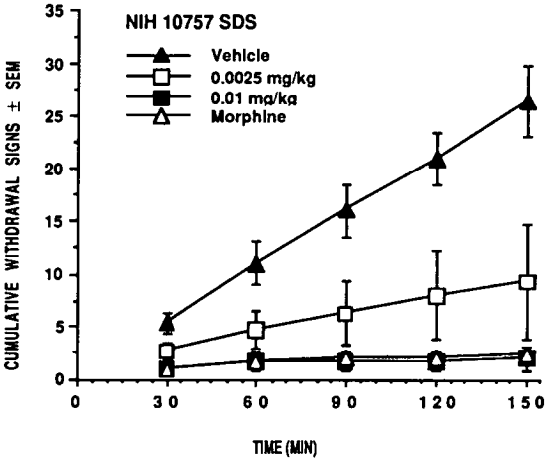
1) TF - 3.6×10^{-3} (1.3×10^{-3} - 9.9×10^{-3})
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.3×10^{-3} (6×10^{-4} - 2.9×10^{-2})
4) HP - 6.5×10^{-3} (4.0×10^{-3} - 1.1×10^{-2})[a]

One mouse died at 30.0.

Special Test: Naloxone antagonism of NIH 10757 (ED80) in TF, AD50 = 0.03 (0.01 - 0.07)

MONKEY DATA
(SDS)

As shown in the fig., this drug substituted completely for morphine. The drug is estimated to be 300 x more potent than morphine. Jaw and body sag and scratching were noted at the higher dose indicating that this dose was higher than that required to substitute for morphine. Onset of action was prompt and duration of action was at least at long as that of the reference standard.

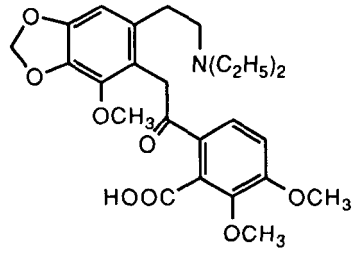


NIH 10757 (±)-*cis*-N-[1-(2-(*trans*-3-Hydroxy-1,2,3,4-tetrahydro)naphthyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride (cont.)

Summary

The evidence indicates that NIH 10757 is a potent mu agonist.

NIH 10759 Ethylnarceine·HCl (6[[6-[2-Diethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3dimethoxybenzoic acid



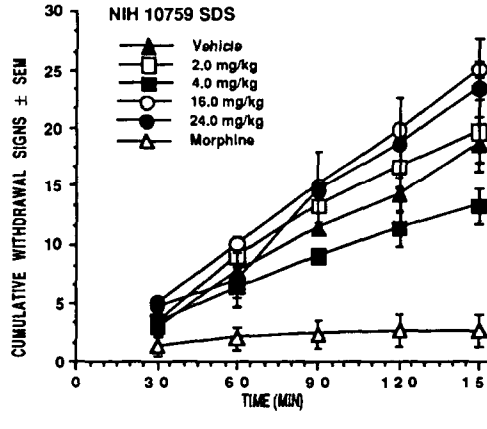
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

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 - 67% at 1.0, 11% at 10.0 and 60% at 30.0
4) HP - Inactive at 1.0, 10.0 and 30.0

MONKEY DATA

SDS

In the dose range of 2.0 - 24.0 mg/kg, NM 10759 did not substitute for morphine. The drug augmented the number of withdrawal signs at the two higher doses. Specifically, the frequency of signs designated wet-dog shakes, vocalizes when abdomen palpated and rigid abdominal muscles was increased.



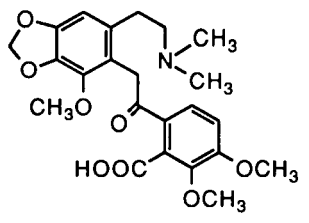
NIH 10759 Ethylnarceine·HCl (6-[[6-[2-Diethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3-dimethoxybenzoic acid (cont.)

Summary

This compound appeared to be free of significant mu agonist properties. The augmentation of withdrawal signs at higher doses could reflect some weak mu antagonist effects.

- -

NIH 10760 Narceine (6-[[6-[2-Diethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3-dimethoxybenzoic acid



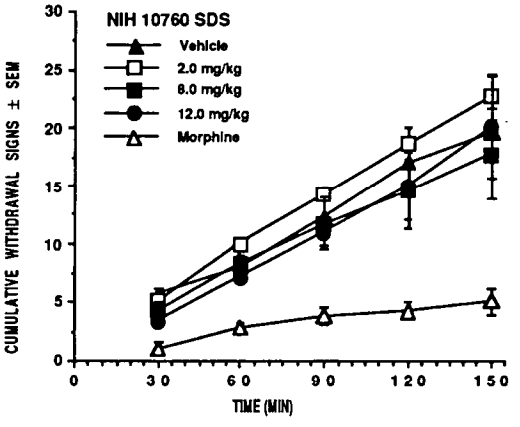
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 6% at 1.0, 11% at 10.0 and 60% at 30.0
4) HP - Inactive at 1.0, 10.0 and 30.0

MONKEY DATA

SDS

As shown in the figure designated NIH 10759 SDS, this compound neither substituted for morphine nor exacerbated. withdrawal in the dose range of 2.0 - 12.0 mg/kg.

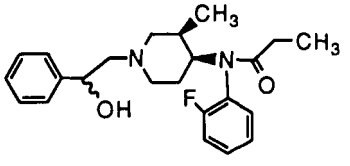


NIH 10760 Narceine (6-[[6-[2-Diethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3-dimethoxybenzoic acid (cont.)

Summary

NIH 10760 did not produce actions suggestive of opioid-like activity.

NIH 10761 (±)-cis-N-[1-(2-Hydroxy)-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(2-fluorophenyl)propanamide hydrochloride



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

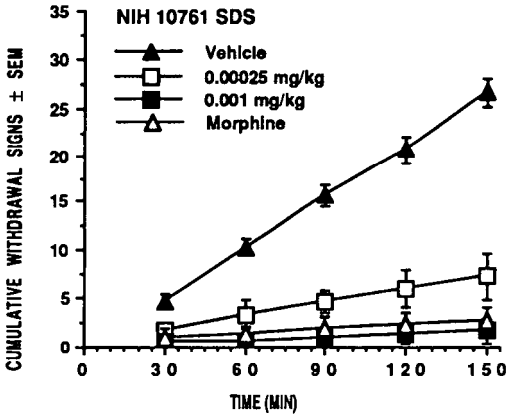
1) TF - 4.2 x 10^{-4} (2.1 x 10^{-4} - 8.3 - 10^{-4})
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.3 x 10^{-4} (5 x 10^{-5} - 3x10-^{4})
4) HP - 3.9 x 10^{-4} (1.9 x 10^{-4} - 8.2 x 10^{-4})

Special Test: Naloxone antagonism of NIH 10761 (ED80) in TF, AD50 = 0.04 (0.01 - 0.10)

MONKEY DATA
SDS

As shown in the accompanying fig., NM 10761 substituted completely for morphine. At the high dose, body and jaw sag, ataxia, eyelid ptosis, and scratching were noted. This indicates that this dose was more than required for substiution. Potency is estimated at 6000 x morphine. Onset and offset of action were similar to those of morphine.

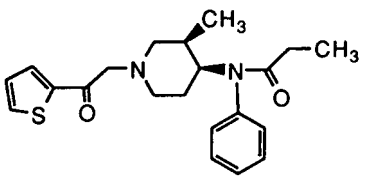
NIH 10761 (±)-*cis*-N-[1-(2-Hyroxy)-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(2-fluorophenyl)propanamide hydrochloride (cont)



Summary

This compound has very potent mu opioid agonist properties.

NIH 10762 (±)-*cis*-N-[3-Methyl-1-[2-oxo-2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenylpropanamide hydrochloride



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 0.03 (0.01 - 0.07)
2) TF vs. M - Inactive at 1.0. 10.0 and 30.0
3) PPQ - 0.016 (0.006 - 0.045)
4) HP - 0.24 (0.12 - 0.50)

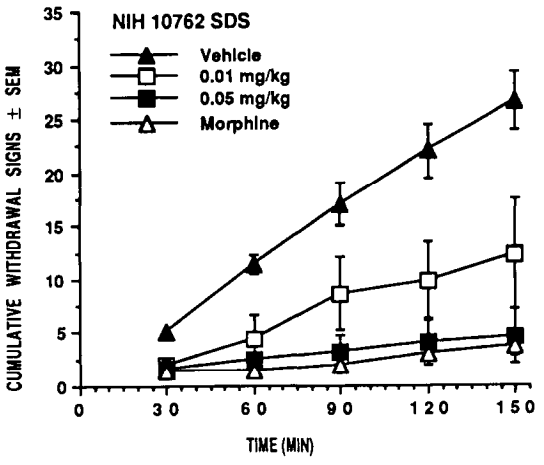
Special Test: Naloxone antagonism of NM 10762 (ED80) in TF, AD50 = 0.03 (0.01 - 0.07;

NIH 10762 (±)-*cis*-N-[3-Methyl-1-[2-oxo-2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenylpropanamide hydrochloride (cont.)

MONKEY DATA
(SDS)

NIH 10762 dose-dependently substituted completely for morphine. Potency estimate is 60 x morphine. Onset and offset of action were similar to morphine.

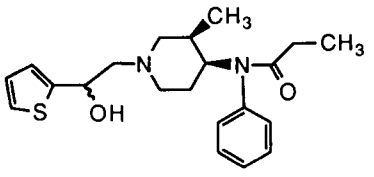
NIH 10762 (±)-*cis*-N-[3-Methyl-1-[2-oxo-2-(2-thienyl)ethyl]-4-piperidinyl]-N-phenylpropanamide hydrochloride (cont.)



Summary

This compound shares many pharmacological effects associated with mu opioid agonists.

NIH 10763 (±)-*cis*-N-[1-[2-Hydroxy-2-(2-thienyI)ethyl]-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



MOUSE DATA ED50 OR AD50
(95% C.L.) or % change (mg/kg)

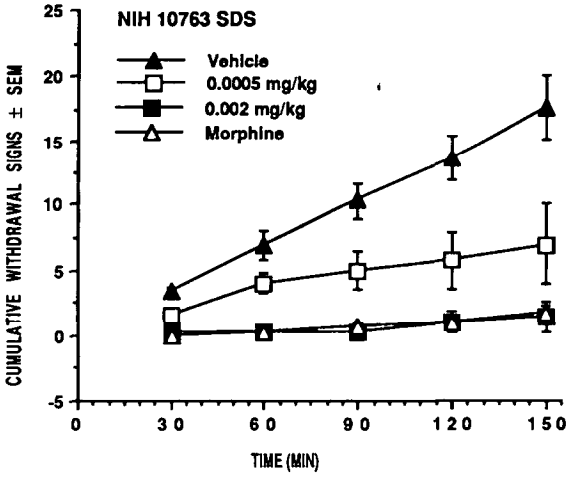
1) TF - $9.6x10^{-4}$ ($4.7x10^{-4}$ - 2x10-^{3})
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ -$1.1x10^{-4}$ ($5x10^{-5}$ - 3x10-4)
4) HP - $1.7x10^{-3}$ ($6.2x10^{-4}$ - $4.5x10^{-3}$)

Special Test: Naloxone vs NIH 10763 ED80 in TF: AD50 = 0.16 (0.08 - 0.32)

MONKEY DATA
(SDS)

NIH 10763 produced a dose-related reduction in withdrawal signs in abruptly with&awn monkeys (see fig). In addition, the drug substituted completely for morphine. Onset and offset of actions were similar to those of the morphine control. Potency estimate is 1500 x morphine.

NIH 10763 (±)-*cis*-N-[1-[2-Hydroxy-2-(2-thienyl)ethyl]-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride (cont.)

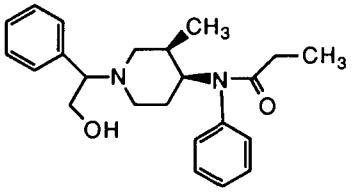


Summary

This compound is a potent mu agonist.

- -

NIH 10765 (±)-*cis*-N-[1-(2-Hydroxy-1-phenylethyl)-3-methyl-4-piperidinel]-N-phenylpropanamide hydrochloride



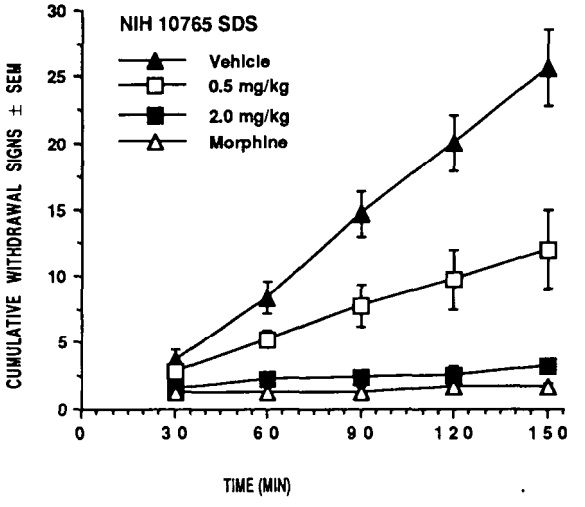
MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - 3.0 (1.3 - 7.2)
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.1 (0.03 - 0.6)
4) HP - 3.4 (1.1 - 10.4)

MONKEY DATA
(SDS)

As shown in the fig., NIH 10765 dose-dependently substituted completely for morphine. The drug behaved like the reference compound, morphine, regarding onset and offset of action. Potency estimate - 1.5 x morphine.

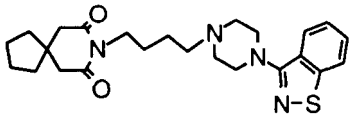
NIH 10765 (±)-*cis*-N-[1-(2-Hydroxy-1-phenylethyl)-3-methyl-4-piperidine1]-N-phenylpropanamide hydrochloride (cont.)



Summary

This drug appears to be morphine-like regarding profile of activity.

NIH 10766 8-[4-[4-(1,2-Benzoisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro(4.5)decane-7,9-dione hydrochloride



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

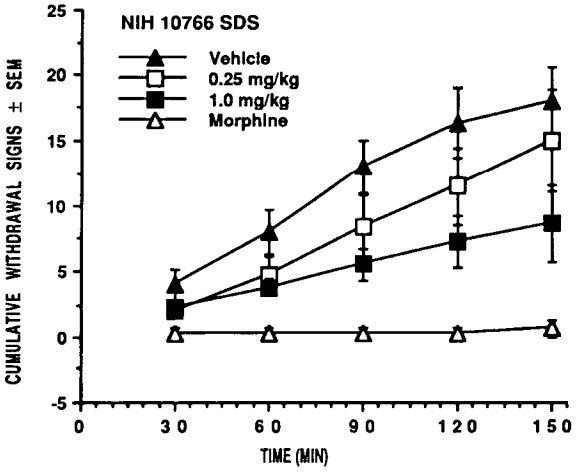
1) TF- 11% at 1.0, 22% at 10.0 and 0% at 30.0[a]
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.07 (0.03 - 0.15)
4) HP - 5.3 (1.9 - 15.1)[a]

[a]At 30.0 mg/kg, the mice showed splayed legs, eyelid ptosis and reduced spontaneous activity.

NIH 10766 8-[4-[4-(l,2-BenzoisothiazoI-3-yl)-1-piperazinyl]butyl]-8-azaspiro(4.5)decane-7,9-dione hydrochloride (cont.)

MONKEY DATA
(SDS)

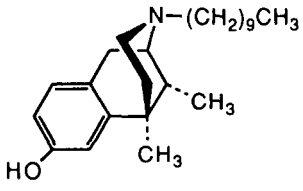
This compound attenuated, but did not completely suppress withdrawal at doses of 0.25 and 1.0 mg/kg (see fig). It reduced the incidence of the signs designated retching, vomiting and fighting. Although the animals at the high dose also had relaxed abdominal muscles and did not vocalize when their abdomens were palpated, many of the vehicle controls also did the same. Therefore, no definite statement can be made with regard to the abdominal responses. However, at the high dose other overt signs were noted such as sagging, slowing, ataxia, eyelid ptosis, and cataleptic behavior.



Summary

The compound has biological activity. The data preclude a definite statement regarding mu and/or kappa-like properties.

NIH 10768 (-)-2-Decyl-5,9α-Dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a,b]
2) TF vs M - Inactive at 1.0, 10.0 and 30.0[a,b]
3) PPQ - 0% at 0.3, 31% at 1.0, 14% at 10.0 and 29% at 30.0[a,b]
4) HP - Inactive at 1.0, 10.0 and 30.0[a,b]

[a]Vehicle propylene glycol and Tween 80
[b]eyelid ptosis

MONKEY DATA

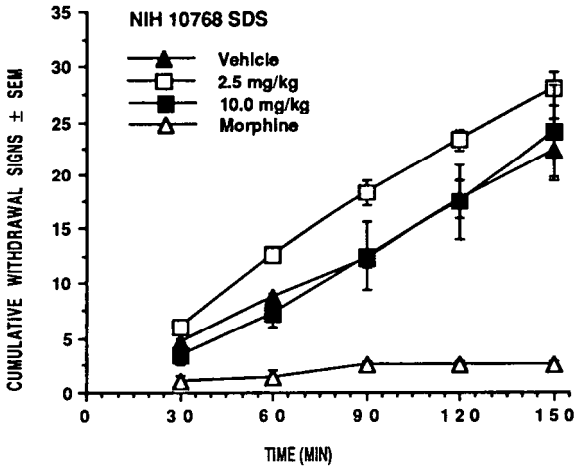
A. Preliminary Study (SDS)

After at a cumulative dose of 7.0 mg/kg (1.0, 2.0 and 4.0 mg/kg each at 15 m intervals respectively), the monkey retched and vomited vigorously. Regular dose of morphine (3 mg/kg) was given to terminate withdrawal. Onset of action for morphine seemed delayed. Vehicle was 25% hydroxypropyl-ß-cyclodextrin in water.

B. Monkey (SDS)

NIH 10768 did not substitute for morphine at doses of 2.5 and 10.0 mg/kg (see fig). At the highest dose, the drug may have exacerbated withdrawal. Vehicle was 25% hydroxypropyl-ß-cyclodextrin in water.

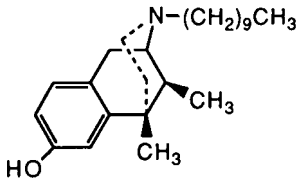
NIH 10768 (-)-2-Decyl-5,9α-Dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide (cont.)



Summary

In morphine-dependent monkeys, 10768 may have mu antagonist properties.

NIH 10769 (+)-2-Decyl-5,9 α-Dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide



MOUSE DATA-ED50 OR AD50 (95% C.L.) or % change (mg/kg)

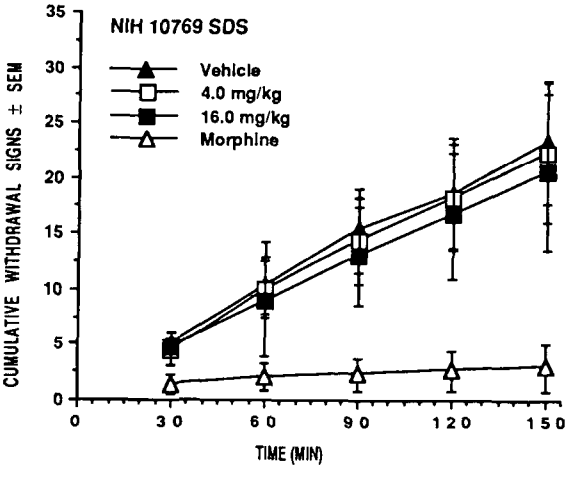
1) TF - 16% at 1.0, 11% at 10.0 and 7% at 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0% at 1.0, 14% at 10.0 and 17% at 30.0[a]
4) HP - 0% at 1.0 and 30.0 and 13% at 10.0

[a]) Vehicle - Propylene glycol, Tween 80 and water.

NIH 10769 (+)-2-Decyl-5,9α-Dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide (cont.)

MONKEY DATA
(SDS)

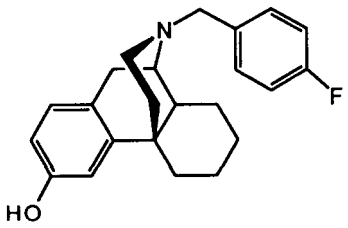
As shown in the accompanying figure, doses of 4 and 16 mg/kg of NIH 10769 neither substituted for morphine nor exacerbated withdrawal. One monkey at the high dose was ataxic and slow. Vehicle was 25% hydroxypropyl-ß-cyclodextrin.



Summary

At the doses tested, NIH 10769 lacked antinociceptive and mu or kappa opioid properties.

NIH 10770 (-)-N-(4-Fluorobenzyl)-3-hydroxymorphinan oxalate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0. 10.0 and 30.0[a]
3) PPQ - 14% at 1.0, 26% at 10.0 and 49% at 30.0[a]
4) HP - 13% at 1.0, 13% at 10.0 and 25% at 30.0[a]

[a]Vehicle - Tween 80, propylene glycol and water

Summary

The drug has very weak antinociceptive properties in the PPQ and HP tests.

NIH 10771 (-)-3-Hydroxy-N-(4-nitrobenzyl)morphinan oxalate

MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

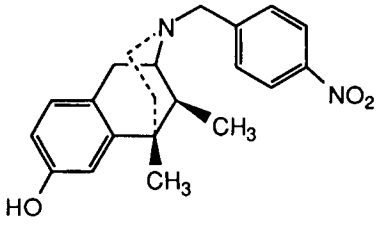
1) TF - 9% at 1.0, 14% at 10.0 and 15% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 14% at 1.0, 14% at 10.0 and 23% at 30.0
4) HP - Inactive at 1.0 and 10.0, 13% at 30.0

[a]Tween 80, propylene glycol and water

Summary

The compound displays very weak, if any, aminociceptive activity.

NIH 10772 (+)-5,9α--Dimethyl-2'-hydroxy-2-(4-nitrobenzyl)-6,7-benzomorphan oxalate



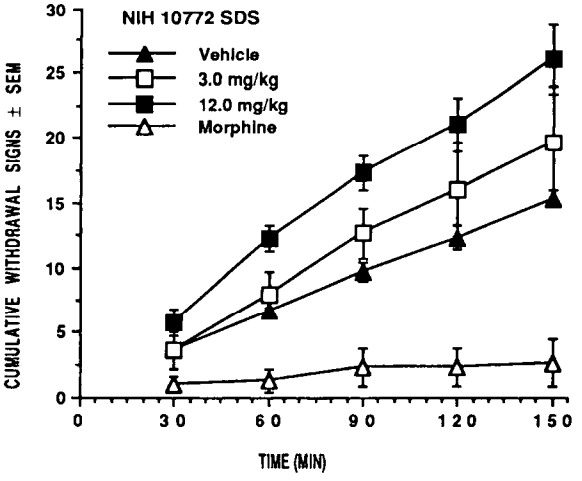
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 10.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0% at 1.0, 14% at 10.0 and 23% at 30.0[a]
4) HP - Inactive at 10.0 and at 30.0[a] 13% at 1.0

[a]) Vehicle - Propylene glycol, Tween 80 and water.

MONKEY DATA
(SDS)

Although the data illusrated in the figure (NIH 10772-SDS) suggested that this compound had exacerbated withdrawal, the results reflected a statistical anomaly. For the first time in 20 years, all the vehicle controls did not vocalize when their abdominal muscles were palpated. Also, all had relaxed abdominal muscles. The result was low withdrawal scores for the controls. However, the monkeys displayed all the other withdrawal signs. It is concluded that NIH 10772 neither exacerbated nor suppressed withdrawal. Vehicle was 25% hydroxypropyl-ß-cyclodextrin in water.

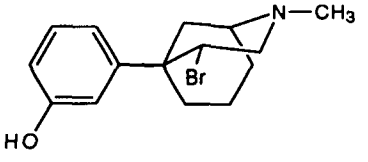


NIH 10772 (+)-5,9α-Dimethyl-2'-hydroxy-2-(4-nitrobenzyl)-6,7-benzomorphan oxalate (cont.)

Summary

Except for some weak activity in the PPQ test, NIH 10772 lacked significant mu or kappa opioid activity.

NIH 10778 4-Bromo-5-(3-hydroxyphenyl)-2-methylmorphan hydrobromide



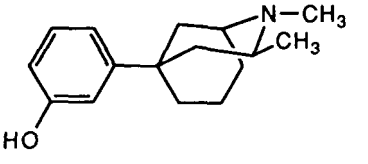
MOUSE DATA-ED50 OR AD50
95% C.L.) or % change (mg/kg)

1) TF - inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 14% at 1.0, 9% at 10.0 and 11% at 30.0[a]
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

[a]Vehicle 5% Tween 80 in water

Summary NIH 10778 is probably devoid of aminociceptive and mu antagonist activity.

NIH 10779 2,3-Dimethyl-5-(3-hydroxyphenyl)morphan hydrobromide



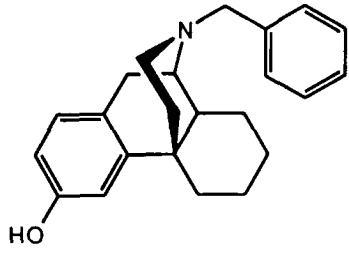
MMOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - 1.3 (0.5 - 3.2)
2) TF vs M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.6)
4) HP - 4.8 (1.9 - 12.4)

Summary

This compound displays an antinociceptive profile of activity not unlike that of morphine.

NIH 10780 (-)-N-Benzyl-3-hydroxymorphinan hydrochloride



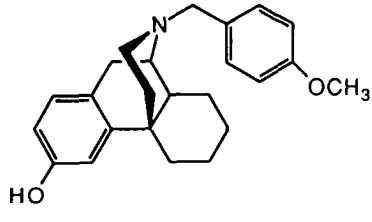
MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 6% at 1.0 and 10.0, 14% at 30.0
4) HP - Inactive at 1.0, 10.0 and 30.0[a]

Summary

NIH 10780 does not show significant antinociceptive or mu antagonist properties.

NIH 10781 (-)-3-Hydroxy-N-(4-methoxylbenzyl)morphinan hydrobromide



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

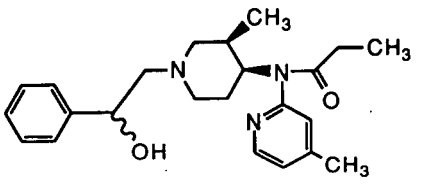
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 13% at 1.0, 57% at 10.0 and 46% at 30.0[a]
4) HP - Inactive at 10.0 and 30.0 25% at 10.0

[a]Vehicle 10% Tween 80 in water

Summary

Some weak antinociceptive activity may be associated with NIH 10781. In addition, it lacks mu antagonist properties.

NIH 10783 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(4-methyl-2-pyridinyl)propanamide oxalate



MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

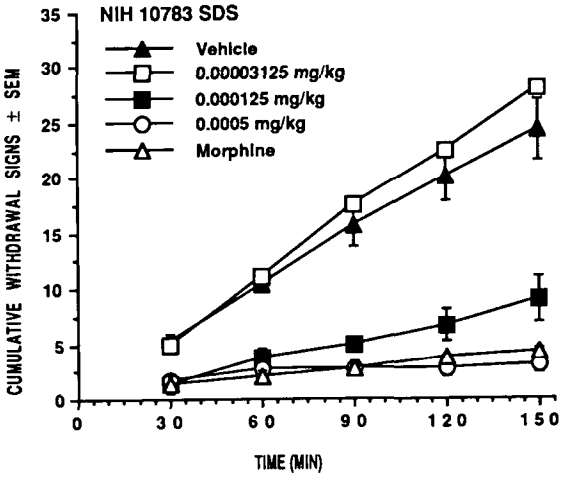
1) TF - 0.002 (0.001 - 0.007)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.0003 (0.0001 - 0.0006)
4) HP - 0.009 (0.005 - 0.015)

[a]) Straub tail at 1.0 Loss of righting reflex at 6.0 and 2 of 6 mice died at 10.0.

Special Test: Naloxone AD50 vs NIH 10783 ED80 in TF = 0.04 (0.04 - 0.1)

MONKEY DATA
(SDS)

NIH 10783 dose-dependently suppressed withdrawal. (see fig. NIH 10783-SDS). Onset of action was rapid and offset was longer than that of morphine at the highest dose, i.e., 0.0005 mg/kg, the monkeys did not require the usual noontime injection of morphine. Potency estimate is 12,000 X morphine.



NIH 10783 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(4-methyl-2-pyridinyl)propanamide oxalate (cont.)

Summary

The profile of activity suggested that this drug has potent mu agonist properties and that the duration of action is longer than that of morphine.

NIH 10799 Dynorphin (2-17) Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Try-Asp-Asn-Gly

MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF-[a]
2) TF vs M -[a]
3) PPQ-[a]
4) HP-[a]

[a]Not tested

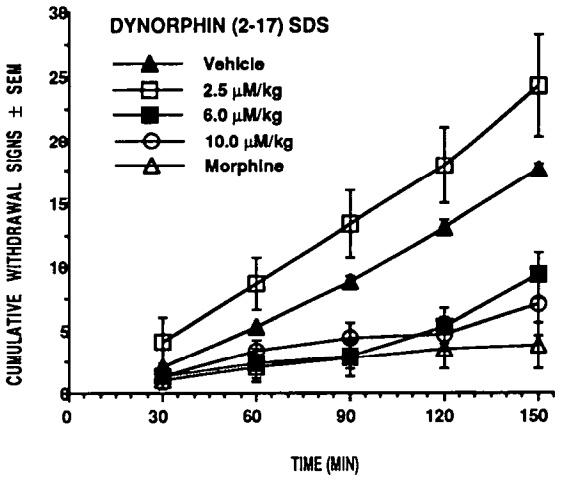
MONKEY DATA
(SDS)

Previous studies in this laboratory demonstrated that dynorphin-(1-13) or Dyn-(1-13) as well as Dyn-(1-10), but not Dyn-(1-8) Dyn-(1-6) and α-endorphin, suppressed withdrawal behavior in maximally dependent rhesus monkeys (Aceto et al, Eur. J. Pharmacol. 83, 1982 and Aceto et al., Fed. Proc. 42, 1983). In order to extend these findings and to determine whether or not deletion of the tyrosine (tyr)-residue was essential for suppression of withdrawal in our animal model, the present studies were initiated. As shown in the fig below, an intravenous dose of 10 μM of Dyn-(2-17) suppressed withdrawal behavior.

Summary

These results confirm that the tyr-residue is not critical for activity in the Dyn series(Takemori and Lee, personal communication) and are in accord with previous observations that short peptide fragments such as Dyn-(1-6) and Dyn-(1-8) are inactive or less potent. They also suggest potential clinical uses for this and other active Dyn peptides in the pharmacotherapy of pain, and tolerence to and dependence on opioids.

NIH 10799 Dynorphin (2-17) Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Try-Asp-Asn-Gly (cont.)



NIH 10800 Pro-Leu-Gly-Amide (MIF)

MOUSE DATA-ED50 OR AD50
(95% C.L.) or % change (mg/kg)

1) TF-
2) TFvsM-
3) PPQ-
4) HP -

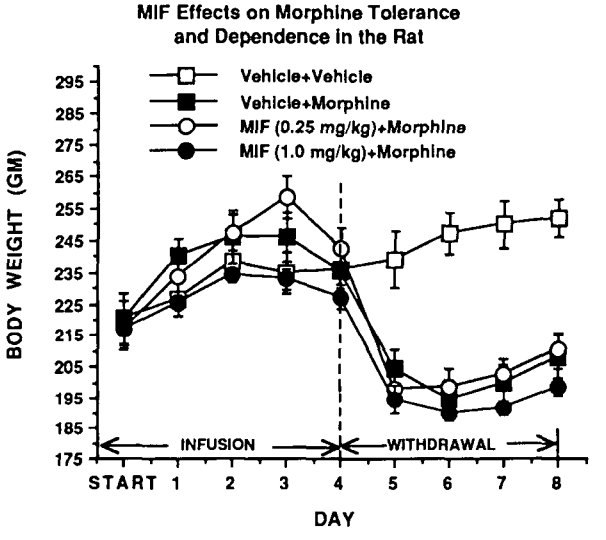
A number of studies have shown that melanotropin release-inhibiting factor (MIF) prevented the development of tolerance to the analgesic, cataleptic, hypothermic and/or hyperthermic actions of morphine, buprenorphine,. and ß-endorphin (Bhargava and Ramarao, Peptides, 10, 767, 1989); Bhargava, NIDA Res. Monog. 70, 337, 1986. However, two other laboratories could not confirm these effects on tolerance to morphine-induced antinociception. Mucha and Kalant, J. Pharm. Pharmacol., 31, 572, 1989 and McLaughlin, et al., Behavioral Neuroscience, 103, 447, 1989.

Because of the possible implications regarding MIF in the pharmacotherapy of opioid abuse, we decided to study the peptide in 2 animal models of morphine tolerance and dependence. Supplies for the rat study were generously supplied by Dr. Bhargava, at the University of Illinois in Chicago. For the monkey study, the drug was purchased from Sigma Chemical Company, Lot #80H0319.

NIH 10800 Pro-Leu-Gly-Amide (MIF) (cont.)

A. Rat Continuous Infusion Study (PPD)

The results of the effects of MIF on morphine-induced changes in body weight during the development of tolerance and physical dependence and during abrupt withdrawal of morphine are illustrated in the accompanying figures. Experimental details and overt behavioral observations noted during abrupt withdrawal are provided in the table. It is apparent that the daily administration of either 0.25 or 1.0 mg/kg of MIF S.C. during the continuous i.p. administration of morphine for 4 days did not significantly influence the development of tolerance to or dependence on morphine.



B. Monkey Data

PPD Study: Nine experimentally naive rhesus monkeys of both sexes, in the weight range of 2.3 - 3.1 kg served as subjects. They were randomly assigned to receive either saline and morphine or MIF at (1.0 or 4.0 mg/kg) and morphine. Each animal was injected S.C. every 6 h with 3 mg/kg of morphine sulfate. In addition, at 10 A.M. each animal received an injection of the assigned dose of saline or MIF. Two hours after the noon injection of morphine, they were scored for the presence or absence of the signs designated scratching, jaw and body sag, slowing and eyelid ptosis during a 15-m observation period. On day 14, each animal was given 0.25 mg/kg s.c. of naloxone·HCl. The animals were scored for any and all withdrawal signs noted. They were designated: lying on side or abdomen, fighting, avoids contact, vocalizes, crawling, restlessness, drowsy, tremors, retching, vomiting, coughing, rigid abdominal muscles, vocalization when abdomen palpated, masturbation, and yawning. For full details consult Aceto et al (Pharmacology 15, 1, 1977).

Table. Effects of Pro-Leu-Gly amide, (MIF), on the Development of Physical Dependence in the Bat Continously Infused with Morphine for 4 Days and then Abruptly Withdrawn

Treatment regimens (4Days)	Hr Off Drugs			
Figure symbols	24	48	72	96
	Significant Differences in Behavioral Scores[a]			
 1. Vehicle + Vehicle	c	ns[c]	c	ns[c]
 2. Vehicle + Morphine	b	ns[b]	b	ns[b]
 3. MIF + Morphine	b	ns[b]	ns[b]	ns[b]
 4. MIF + Morphine	b	ns[b]	ns[b]	ns[b]

[a]Behavior score: hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing

[b]One-tailed test (Mann-Whitney U-test), $p < 0.05$ compared to vehicle controls, ns - not significant

[c]One-tailed test (Mann-Whitney U-test), $p < 0.05$ compared to morphine controls, ns - not significant

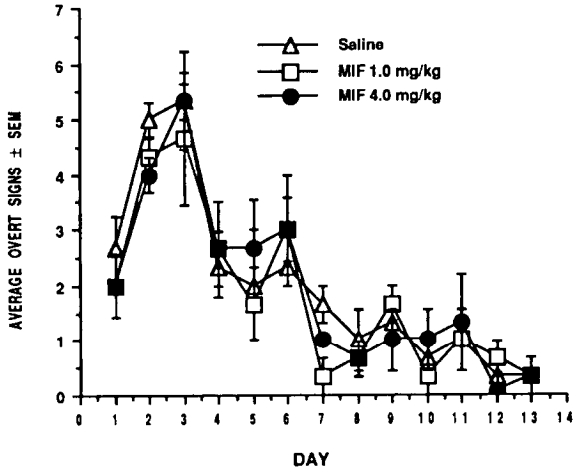
[d]Vehicle was water. Days 1-4: 0.1 ml/l00 g body weight s.c., one injection/24 hr and 8 ml i.p./24 h; N=4

[e]Vehicle s.c., injection as above. Morphine sulfate i.p. 50 mg/kg on day 1; 100 mg/kg on day 2; 200 mg/kg on days 3 thru 4; N=6

[f]MIF s.c injection/24 hr; 0.25 mg/kg on days 1 thru 4 and morphine sulfate i.p. as above; N=5

[g]MIF s.c. one injection/24 hr. 1.0 mg/kg on days 1 thru 4 morphine sulfate i.p/ as above; N=5

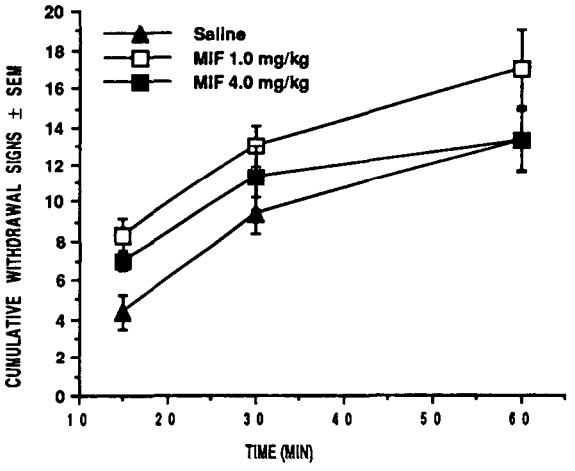
Effect of NIH 10800 (MIF) on the Development of Tolerence to Morphine In Rhesus Monkeys



As shown in the fig. (Effects of MIF or Saline on Tolerance to Morphine), the results of the saline-treated and MIF-treated animals are indistinguishable. By day 9, tolerance to morphine was evident. Had tolerance not developed in the MIF-treated monkeys, they would have continued to show the overt signs initially seen in naive subjects first challenged with morphine.

Regarding physical dependence, MIF did not prevent the development of physical dependence on morphine. Naloxone precipitated withdrawal and as illustrated in the fig. (Naloxone challenge of morphine-dependent monkeys treated daily with MIF).

Naloxone Challenge of Morphine-Dependent Rhesus Monkeys Treated with NIH 10800 (MIF)



Summary Results in rats and monkeys indicate that MIF does not influence the course of the development of tolerance to and/or physical dependence on morphine.

ACKNOWLEDGEMENTS

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BIOLOGICAL EVALUATION OF COMPOUNDS FOR THEIR PHYSICAL DEPENDENCE POTENTIAL AND ABUSE LIABILITY. XVII. DRUG EVALUATION COMMITTEE OF THE COLLEGE ON PROBLEMS OF DRUG DEPENDENCE, INC. (1993)

A. E. Jacobson

PURPOSES OF THE DRUG EVALUATION COMMITTEE

The Drug Evaluation Committee (DEC) of the CPDD (Dr. T. Cicero, Chairman) is charged with the responsibility of determining the physical dependence potential and abuse liability of potential analgesics, stimulants and depressants, and with associated methodological research. The drugs are obtained from investigators in universities, industrial groups, and the public sector. The testing function is carried out under the auspices of the CPDD as a public service and has provided information to pharmaceutical industry and governmental agencies for the appropriate scheduling of a drug with the potential for abuse. The information which DEC provides to university researchers, who frequently work under a NIDA grant, is useful for determining the desirability of structural modification of a drug and the DEC biological data are often needed for publication of their work in medicinal chemistry journals.

THE DRUG EVALUATION COMMITTEE'S PARTICIPATION IN THE MAIN FUNCTIONS OF CPDD

The dissemination of information on the physical dependence potential and abuse liability of drugs for the public welfare continues to serve one of the major purposes of the CPDD, as it has since the establishment of CPDD as a Committee of the National Academy of Sciences, National Research Council. The data obtained by the DEC have been requested this past year by U.S. governmental agencies, such as NIDA, the Drug Enforcement Agency (DEA), and the Food and Drug Administration (FDA). Examples of our continued cooperation with governmental agencies are as follows.

1) We were asked this year to examine a drug seized by the DEA, and its structural relative. These compounds (see NIH 10759 and 10760, in table 9) were not found to have opioid-like effects.

2) Data recently obtained on LAAM (levo-alpha-acetylmethadol) by the DEC were provided to the FDA in response to the request of one of their officials. These data were apparently required as part of the comprehensive information necessary for a scheduling decision on LAAM.

3) Some of our older data were also utilized by the FDA. In 1982 the analgesic testing groups reported on a drug which was recently found to be a minor contaminant in a commercially marketed product. These data were used to allow retention of the commercial product on the market. Toxicological studies on the contaminant will be requested from the supplier by the FDA.

4) Evaluation of NIH 10710 (table 8) and 10766 (table 9) by the analgesic testing groups, was requested by the NIDA Medications Development group. The testing of NIH 10710 by the stimulant/depressant groups was reported last year (as CPDD 0037, Woolverton et al. 1993).

5) Lastly, the stimulant/depressant groups evaluated two compounds at the request of NIDA, CPDD 0039 and 0040. The World Health Organization (WHO) asked NIDA to provide data on the latter compound, Mesocarb (CPDD 0040, table 10).

Thus, the DEC in this past year has provided data for NIDA, the FDA and DEA, and the WHO. The CPDD has then, through the DEC. fulfilled one of its main functions, providing essential dependence potential and abuse liability information on drugs with the potential for abuse to governmental agencies, pharmaceutical industry, and university researchers.

PUBLICATION OF DATA

The CPDD's evaluation of drugs began around 1951 and the data gathered on these drugs have been published since 1979 in a NIDA Monograph "Problems of Drug Dependence". The published data have always been archival, in the sense that they were declared to be reference material. In 1977 and 1978, the testing data were published by CPDD as part of the proceedings of the annual scientific meeting. From 1951 to 1976, however, the reports were published by the National Academy of Sciences. In those volumes the proceedings of the CPDD meeting were not archival and were not meant to be used as reference material, but rather to record research in progress. The drug testing results, however, were available for quotation. Thus, for more than 40 years, the results obtained from drug testing have been part of the open literature. Unfortunately the volumes published by the National Academy of Sciences are out of print and have been unavailable to all but the few individuals who have maintained a private library of such work. Fortunately, most of these data have now been included in a computerized database by the NIDA Medications Development group, and this database can be accessed and utilized by researchers.

GROUPS REPRESENTED IN THE DEC AND THEIR FUNDING

The testing function has evolved over the past 40 years. At this time two university groups, one at the Medical College of Virginia (MCV) of Virginia Commonwealth University (headed by Drs. M. Aceto and L. Harris) and the other at the University of Michigan (UM) Medical School (led by Dr. J. Woods), are involved with testing potential analgesics, and three groups, one at each of the above mentioned medical schools (Dr. G. Patrick at MCV, and G. Winger at UM) and the third at the University of Chicago Medical School, have been involved with work on stimulants and depressants. Dr. W. Woolverton, who is in charge of the latter group, will continue the work in the future at the University of Mississippi Medical Center. The stimulant/depressant work by the consortium of university groups is carried out through a NIDA grant to Washington University under the direction of Dr. T. Cicero, the Chairman of the Drug Evaluation Committee. The analgesic work at the Medical College of Virginia is carried out under a contract with NIDA, and the work at the University of Michigan Medical School is pursued with a NIDA grant. Each of these groups receives a supplemental grant from the CPDD for their research. There are eight members of the Drug Evaluation Committee, one from each of the five testing groups, Dr. Cicero, myself as

Biological Coordinator, and Dr. Steve Holtzman, who is also a member of the Board of the CPDD. Three representatives from NIDA also attended the annual meeting of the Drug Evaluation Committee.

PROCEDURES FOR EVALUATION OF DRUGS

The analgesic testing program employs some or all of the following assays:
1) Antinociceptive and narcotic antagonist assessment in the mouse.
2) Substitution for morphine and primary physical dependence by rat infusion.
3) Single dose suppression and, if warranted, precipitated withdrawal, as well as primary physical dependence studies in the rhesus monkey.
4) Opioid receptor binding.
5) Electrical stimulation of the mouse vas deferens.
6) Self-administration in the rhesus monkey.
7) Drug discrimination in the rhesus monkey.
8) Analgesic studies in the rhesus monkey.
9) Respiratory function studies in the rhesus monkey.

The stimulant and depressant groups *use* the following methodology:
1) Inverted screen test and spontaneous locomotor activity, in mice.
2) Physical dependence potential by substitution in pentobarbital-dependent rats using continuous intraperitoneal infusion.
3) Primary physical dependence determination in rats, by infusion.
4) Self-administration studies in rhesus monkeys.
5) Drug discrimination studies in rhesus monkeys.

A complete description of each of the tests on potential analgesics can be obtained from the reports from the Medical College of Virginia (Aceto et al. 1994) and the Universitv of Michigan (Woods et al. 1994). The procedures used for the evaluation of stimulants and depressants are described in the group report which will be written this year by Dr. Graham Patrick (Patrick et al. 1994). All of these testing procedures were summarized in a previous report (Jacobson 1993).

STATISTICS

About 62 compounds were evaluated as potential analgesics this year at either or both the Medical College of Virginia and the University of Michigan. Several others were explored as basic research topics (Aceto et al. 1994) and do not appear in the tables of this report. For example, researchers at the Medical College of Virginia investigated the effects of various dynorphins (2-17, 1-13, and a three amino acid peptide MIF), as well as (-)-nicotine). Of the compounds which were sent for testing, 5% came from industrial sources (domestic and foreign), 48% came from university groups (domestic and foreign), 14% were sent by government agencies, and the remaining 32% came from a non-profit institution. Except for the diminution of requests for examination of drugs from industrial sources, a downward trend observed over the last decade, and the large number from a single group, neither the sources of the drugs nor their number were particularly unusual.

SURVEY OF EVALUATED COMPOUNDS

1) Analgesics

In order to more easily discern the biological effect of structural changes in a basic molecular structure, the examined drugs were grouped in structural classes in the

following 10 tables. More comprehensive data on the individual drugs can be obtained from the reports of the members of the DEC (Aceto et al. 1994, Woods et al. 1994).

Two 4,5-epoxymorphinans and two phenylmorphans are shown in table 1, and eight morphinans, divided between the (+)- and the (-)-enantiomeric series are listed in table 2. Table 3 displays eight N-substituted benzomorphans, also divided between the (+)- and the (-)-enantiomeric series. Three arylpiperidines are shown in table 4, and 18 compounds related to fentanyl are listed in tables 5-7. The remaining two tables consider the miscellaneous compounds.

Remarkable differences in the activity of enantiomers can be seen among the fentanyl compounds. For example, there is about a 16,000-fold difference in the tail flick assay between the enantiomeric NIH 10743 and 10744 (table 5). They are 10,000-fold different in the vas deferens preparation and 10744 was noted to exhibit only partial agonist properties. NIH 10744 does not substitute for morphine in the SDS assay in monkeys. Similarly, the enantiomers NIH 10741 and 10742 in table 5 show even more extreme differences in the vas deferens preparation (4,000,000-fold different), and appear to be 10,000 to 20,000-fold different in the SDS assay. One of these fentanyl compounds, NIH 10741, was found to be 20,000 to 50,000 times more potent than morphine in the SDS assay. The potency of the other fentanyl compounds ranged from morphine-like to 12,000 times more potent than morphine in that assay. The extremely potent drugs are likely to be interesting research tools, especially if they are found to be opioid receptor subtype selective.

An additional compound of interest is NIH 10678 (table 8) which appears to have a novel profile of action, dissimilar to other opioid agonists and partial agonists. Structurally, as a two-dimensional drawing, the compound does not appear to have much resemblance to the usual opioid types.

2) Stimulants and Depressants

The number and origin of stimulants or depressants were not unusual this year. The NIDA grant for this work is mostly utilized for basic research by the individual investigators. Four compounds were released for publication this year. That number of compounds is around the maximum which we are able to examine with current available resources. Two new compounds were received for evaluation (5/1/92 to 4/30/92).

This Monograph contains the detailed report (Patrick et al. 1994) on the four compounds released for publication. These are CPDD 0027 (*cis*-4-phosphomethyl)-2-piperidinecarboxylic acid), 0035 (1.3.4.16b-tetrahydro-2-methyl-2 *H*,10*H*-indolo[2,1-*c*]pyrazino[1,2-a][1,4] benzodiazepine-16-carboxylic acid methyl ester hydrochloride), 0039 (Aminorex hydrochloride [4,5-dihydro-5-phenyl-2-oxazolamine hydrochloride]), and 0040 (Mesocarb [3-(1-methyl-2-phenylethyl)-N-(phenylaminocarbonyl)sydnone imine]). The latter two compounds were submitted by NIDA. The molecular structures of these four compounds and the summarization of the data collected on them by the Stimulant/Depressant testing groups in the Drug Evaluation Committee are shown in table 10.

ABBREVIATIONS USED IN TABLES 1 - 9

Rounded numbers are used in the tables; M = morphine. For precise values and details of the procedures see Aceto et al. 1994 and Woods *et al.* 1994.

For "E" notation: 1E-3 = 1 x 10^{-3} or 0.001 M (1 μ), 1E-6 = 1 μM, 1E-9 = 1 nM, 1E-12 = 1 pM (picomole), and 1E-15 = 1 fM (femtomole).

1) **MOUSE E50/AD50:** Antinociceptive Assays (SC injection)
Confidence limits are listed in the MCV report (Aceto *et al.* 1994).

HP = hot plate (morphine ED_{50} = 0.8 (0.3-1 .8))

PPQ = phenylquinone (morphine ED_{50} = 0.23 (0.20-0.25))

TF = tail-flick (morphine ED_{50} = 5.8 (5.7-5.9))

TFA = tail-flick antagonism vs. morphine (naltrexone AD_{50} = 0.007 (0.002-0.02); naloxone AD_{50} = 0.035 (0.01-0.093)).
I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

2) **IN VITRO** (Data from UM, Woods *et al.* 1994)

RBH = binding affinity in rat cerebrum membranes (displacement of 0.5 nM [^{3}H]etorphine) in the presence of 150mM NaCl (morphine EC_{50} = 23.6).
NE = no effect.
NOTE: Contemporary EC_{50} data cannot be directly compared with those from some previous reports (Jacobson 1985, and preceding years) which were obtained under "-NaCl" (without NaCl) conditions.

VD = electrically stimulated mouse vas deferens EC_{50} values, rounded to one significant figure. Partial agonist indicated by % inhibition of twitch in parenthesis; [A] = antagonism by naltrexone.
SE = slight effect on twitch.
NE = No significant agonist or antagonist effect.
ANT = Antagonist activity. Selective antagonist activity at μ δ, and/or κ receptors is noted in parentheses. The antagonist effect may or may not be competitive.

Compounds which suppress the twitch and are not antagonized by naltrexone or other narcotic antagonists are said to be non-opioid agonists (*e.g.,* clonidine, a non-opioid agonist, can suppress the twitch but is not antagonized by naltrexone). Compounds which bind with reasonable affinity in the RBH assay and do not suppress the twitch in the VD may have narcotic antagonist properties. The opioid receptor at which the drug exerts its antagonist effect is determined by testing various concentrations of the drug to induce a blockade (antagonism) of the suppression of the twitch in the VD preparation caused by sufentanil (μ), DSLET (δ) or U50,488 (κ) (for these data see Woods *et al.* 1994).

3) **IN VIVO:** in the rhesus monkey (from MCV, Aceto *et al.* 1994; prior to 1988 from MCV or UM).

SDS = single-dose-suppression
NS = no suppression
CS = complete suppression
PS = partial suppression
(Parenthesized numbers = dose range studied, in mg/kg)

Other Studies (noted in the footnotes to the tables)
A) In Rat:

RI = rat continuous infusion (data from MCV)
1) **SM** = substitution for morphine
NS = no substitution for morphine
CS = complete substitution
PS = partial substitution

2) **PPD** = primary physical dependence

B) In Rhesus Monkey:

1) **PPt-W** = studies in non-withdrawn monkeys (data from MCV)
PW = precipitated-withdrawal at dose levels, in mg/kg, indicated in parentheses &/or comparison with naloxone [N].
SP = slight precipitation
NP = no precipitation

2) **ND** = studies using non-dependent monkeys (data from MCV)
M-like = morphine-like effect.

3) PPD = primary physical dependence (data from MCV)

4) **SA** or SI = self-administration or self-injection (data from UM)
NE = no effect
High = codeine-like
IN = intermediate between saline and codeine
SE = slight effect

5) **DD** = drug discrimination (data from UM)
NE = no effect
CS = complete substitution

6) **MA** = monkey analgesia (data from UM)

7) **RF** = respiratory function (data from UM)

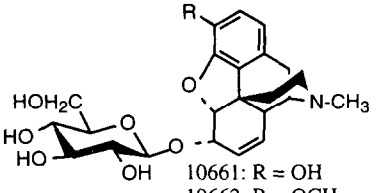
C) In Vitro (data from UM)

BIND - binding affinity using monkey brain cortex membranes (selectivity for μ, κ, and δ opioid receptors using [^{3}H]-sufentanil, [^{3}H]-DPDPE and [^{3}H]-U69,593, respectively).

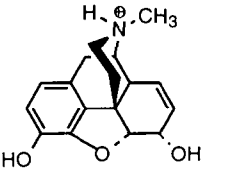
Previous Reports

Previous work on a compound is noted using the year listed in the monograph title (*e.g.,* work cited as "1992" indicates that the work was included in "Problems of Drug Dependence 1992", which was published in 1993). Note that the monograph's publication date may be one year after the titled year of the monograph. Complete details of the original work on a compound can be found in the Annual Report of either Aceto *et al.* or Woods *et al.*

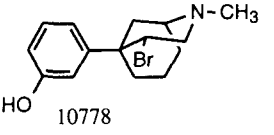
TABLE 1. 4,5-EPOXYMORPHINANS AND 5-PHENYLMORPHANS[a]



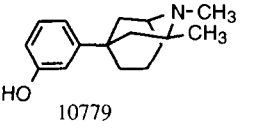
10661: R = OH
10662: R = OCH_3



10753 (Morphine sulfate)



10778

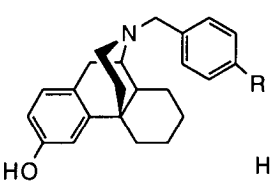


10779

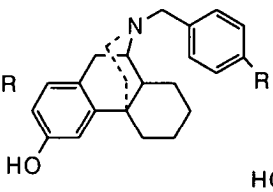
	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10661	-	0.4	0.7	I	40.4 nM	167 nM[b]	CS (0.5,2)[c]
10662	-	I	I	I	6.4 μM	12 μM(80)[d]	PS (3,12)[e]
10753	3.1	0.4	0.73	I	74.5 nM	544 nM[A][f]	CS(2.0)[1 x M][g]
10778	I	I	I	I	753 nM	ANT[h]	-
10779	4.8	0.3	1.3	I	592 nM	15.8 μM[i]	-

a) See text for explanation of column headings and abbreviations.
b) Selective μ-agonist.
c) Prompt action, long duration.
d) Weak κ -agonist.
e) Does not completely substitute for M or exacerbate withdrawal.
f) μ- and κ·-agonist.
g) Prompt action, 2.5 hr. duration.
h) Weak, non-selective antagonist, no agonist activity.
i) Low potency u-partial agonist.

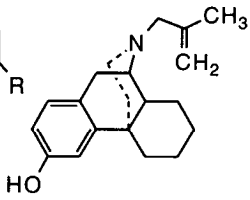
TABLE 2. MORPHINANS[a]



10770: (-) R=F
10771: (-) R=NO_2
10780: (-) R=H
10781: (-) R=OCH_3



10735: (+) R=H
10748: (+) R=F
10749: (+) R=NO_2

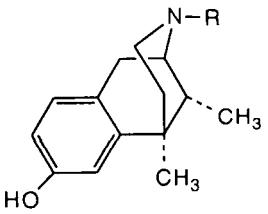


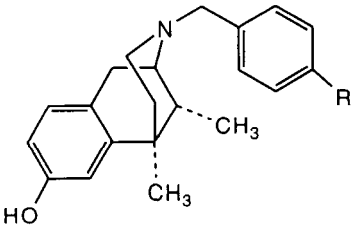
10796 (+)

	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10735	-	-	-	-	>6 μM	ANT[b]	-
10748	I	12	I	I	>6 μM	NE	NS (4,16)
10749	-	-	-	-	>6 μM	Insoluble	-
10770	I	I	I	I	1.3 μM	ANT[c]	-
10771	I	I	I	I	2.1 μM	7 nM(20)[d]	-
10780	I	I	I	I	260 nM	ANT[e]	-
10781	I	I	I	I	>6 μM	5 nM(18)[f]	-
10796	-	-	-	-	>6 μM	ANT[c]	-

a) See text for explanation of column headings and abbreviations.
b) Low potency κ-antagonist activity (and some δ-antagonist activity).
c) No agonist activity, very weak κ--antagonist with limited selectivity.
d) Partial agonist, weak non-selective antagonist.
e) Weak non-selective antagonist.
f) Very weak partial agonist only slightly antagonized by naltrexone, very weak non-selective antagonist.

TABLE 3. 6, 7-BENZOMORPHANS[a]





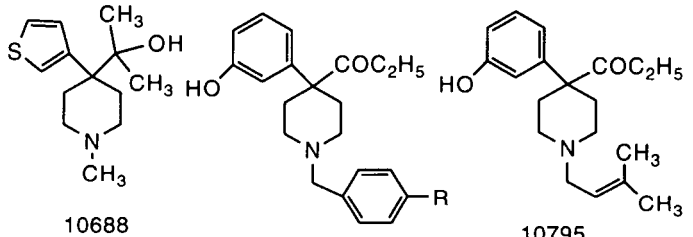
10697: (-) R=$(CH_2)_7CH_3$
10698: (+) R=$(CH_2)_7CH_3$
10768: (-) R=$(CH_2)_9CH_3$
10769: (+) R=$(CH_2)_9CH_3$

10750: (-) R=NO_2
10751: (+) R=F
10752: (-) R=F
10772: (+) R=NO_2

	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH #	HP	PPQ	TF	TFA	RBH	VD	SDS
10697	5.4[b]	0.5[b]	10.0[b]	I[b]	226 nM[b]	ANT[b,c]	NS(1,4)[d]
10698	11.1[b]	7.8[b]	I[b]	I[b]	3.4 μM[b]	ANT[b,e]	NS(4,16)
10750	I	I	I	I	>6 μM	1.9 nM(37)[A][f]	NS(4,16)
10751	I	I	I	I	>6 μM	ANT[g]	NS(4,16)
10752	I	I	I	I	>6 μM	Insoluble	NS(4,16)
10768	I	I	I	I	>6 μM	SE[h]	NS(2.5,10)[i]
10769	I	I	I	I	>6 μM	13 nM(20)[j]	NS(4,16)
10772	I	I	I	I	>6 μM	ANT[k]	NS(3,12)

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1993.
c) Non-typical μ- and κ-agonist, weak μ-antagonist.
d) May exacerbate withdrawal, possible κ-agonist properties.
e) Weak μ- and κ-antagonist.
f) Partial agonist, weak non-selective μ- and κ-antagonist
g) Weak non-competitive κ-antagonist with limited selectivity.
h) Weak low efficacy partial agonist, antagonized by naloxone at lower concentrations; non-opioid action at higher concentrations.
i) May exacerbate withdrawal at 10 mg/kg.
j) Low efficacy partial agonist or non-opioid.
k) Weak non-selective antagonist.

TABLE 4. ARYLPIPERIDINES[a]



10688

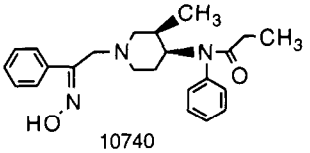
10738: R=NO_2
10739: R=F

10795

	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10688	I[b]	5.4[b]	I[b]	I[b]	10.3μM[b]	SE[b,c]	PS(4,24)[b,d,e,f]
10738	-	-	-	-	3.4μM	ANT[g]	-
10739	-	-	-	-	1.3μM	ANT[h]	-
10795	-	-	-	-	507nM	ANT[I]	-

a) See text for explanation of column headings and abbreviations.
b) Previously reported - 1992.
c) Unusual partial agonist with doubtful opioid action.
d) **RI(SM**: NS, but behavioral suppression; and **PPD**: PS): Doubtful μ-like dependence potential
DD - κ agonist effects (>5.6 mg/kg), no μ agonist or antagonist activity;
MA - 100% effect (10 mg/kg, 50°, attenuated by quadazocine);
RF - decreased function (attenuated by quadazocine);
SA - limited reinforcing capacity.
f) New data - **PPD** (monkeys) - produced physical dependence, probably μ-related and, possibly, κ and/or dopaminergic.
g) Low potency κ- and δ--antagonist; not simple competitive-type.
h) Low potency, mostly δ--antagonist (some k-antagonism); not competitive.
i) Weak, partial agonist, non-opioid; weak non-selective antagonist.

TABLE 5. FENTANYL-LIKE COMPOUNDS[a]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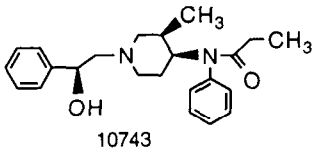
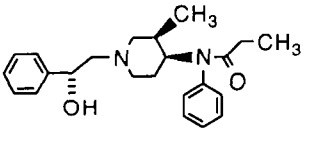
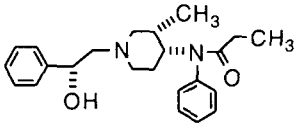

10743


10741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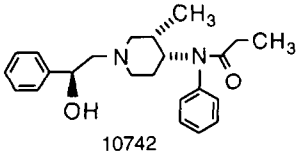
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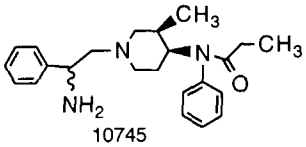
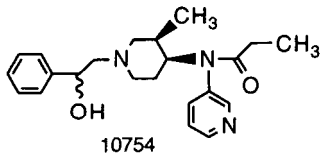

10745

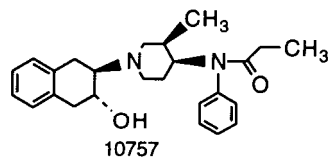
	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	V D	SDS
10740	1.8	0.8	0.9	I	3 µM	2 µM(47)[A][b]	CS(0.3-3.6)
10741	0.0001	9E-5	2E-4[c]	I	5.9 nM	56 fM[A][d]	CS (1.5E-4-3E-5)[e]
10742	0.08	0.03	0.06[c]	I	102 nM	1.4uM[A][f]	CS[30 x M]
10743	0.0013	1.2E-4	8E-4[c]	I	6.8 nM	96 fM[A][g]	CS[1500 x M]
10744	2.1	0.6	13.0	I	380 nM	1nM(28)[A][h]	NS(0.6,12)[i]
10745	0.7	0.09	0.6[c]	I	410 nM	7.6 nM[A]	CS[4-6 x M]

a) See text for explanation of column headings and abbreviations.
b) No significant agonist or antagonist activity.
c) Naloxone (AD50) vs ED_{80} of 10741=0.008, of 10742 and 10745=0.03, of 10743=0.035.
d) Biphasic (also, 4 nM[A]).
e) 20,000-50,000 x M.
f) Biphasic.
g) Biphasic (also, 6.7E-9[A]).
h) Weak partial µ-agonist, and weak µ-antagonist.
i) Biphasic (weak antagonist at lower doses; no effect at highest dose).

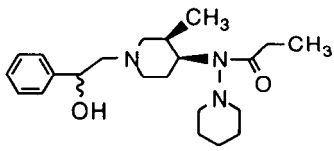
TABLE 6. FENTANYL-LIKE COMPOUNDS (CONTINUED)[a]



10754



10757



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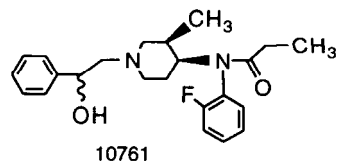

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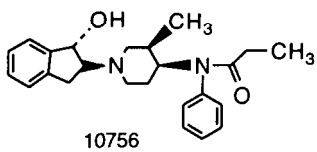

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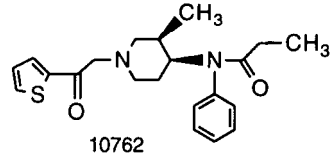
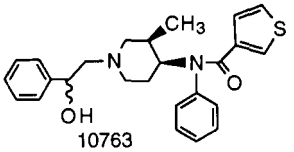


10762

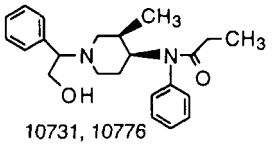
	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10754	0.004	4E-4	0.001	I	28.4 nM	32 nM[A][b]	CS[2000 x M]
10755	0.16	0.07	0.12[c]	I	377 nM	90 nM(85)[A]	CS[10 x M]
10756	0.18	0.03	0.14[c]	I	1037 nM	310nM(91)[A][d]	CS[6 x M]
10757	0.006	1E-3	3.6E-3	I	48.2 nM	Agonist[e]	CS[300 x M]
10761	4E-4	1E-4	4E-4[c]	I	1.02 nM	33 nM[f]	CS[6000 x M]
10762	0.24	0.02	0.03[c]	I	-	-	CS[60 x M]

a) See text for explanation of column headings and abbreviations.
b) Actions at μ- and δ-opioid receptors.
c) Naloxone (AD_{50}) vs ED80 of 10755=0.04, of 10756=0.11, of 10761=0.04, and of 10762=0.03.
d) Partial agonist, u-selective.
e) Fairly potent, multiphasic action.
f) Only slightly antagonized by μ-, δ-, or k-antagonists; possible δ-agonist and μ-antagonist activity.

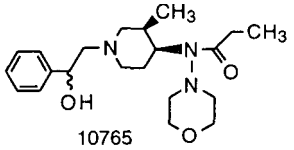
TABLE 7. FENTANYL-LIKE COMPOUNDS (CONTINUED)[a]



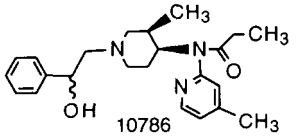
10763



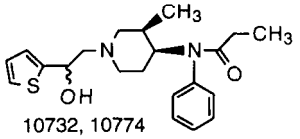
10731, 10776



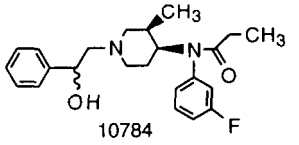
10765



10786



10732, 10774

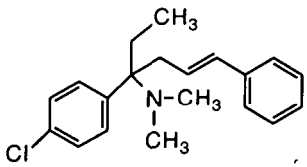


10784

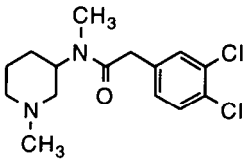
	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10763	2E-3	1E-4	1E-3[b]	I	5.6 nM	113 nM[c]	CS [1500 x M]
10765	3.4	0.1	3.0	I	-	-	CS[1.5 x M]
10732; 10774	0.6[d]	0.3[d]	0.7[d]	I[d]	3 µM[d] 5.3 µM	15.7 µM[d,e] 1.6 µM(85)[A][f]	CS[12 x M][d]
10731; 10776	4E-3[d]	1E-3[d]	3E-3[d]	I[d]	7.3 nM[d] 2.8 nM	300nM[NA][d,g] 3nM[A][h]	CS[750 x M][d]
10783	9E-3	3E-4	2E-3	I	19 nM	7 nM [A][i]	CS [12,000xM]
10784	-	-	-	-	6.5 nM	7.6 nM[A][i]	-

a) See text for explanation of column headings and abbreviations.
b) Naloxone (AD50) vs ED_{80} = 0.02.
c) Slight antagonism by µ- and k-antagonists.
d) Published in 1992.
e) Slight shift by µ- and δ-antagonists; actions possibly non-opioid.
f) µ-selective agonist.
g) Non-opioid action.
h) Potent agonist primarily at δ- and, possibly, p-receptors.
i) Potent µ-agonist.

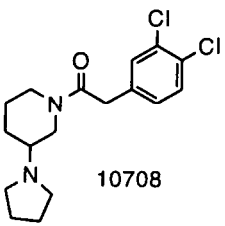
TABLE 8. MISCELLANEOUS[a]



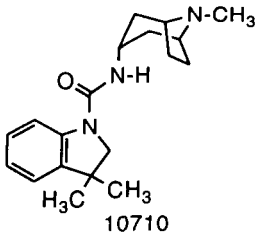
10678



10707



10708



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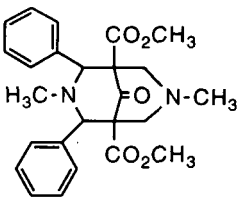


10711: R=Cl
10712: R=OH
10714: R=CH3
10715: R=NO2
10716: R=H

		MOUSE ED50/AD50			IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10678	-	0.9	13.7	I	216 nM[b]	734 nM(90)[A]	CS[0.5 x M][c]
10707	I	5.6	I	I	>6 μM	NE	NS(2.5,10)
10708	-	-	-	-	>6 μM	58 nM(22)	-
10710	I	I	I	I	-	-	NS(2,8)[d,e]
10711	I[f]	I[f]	I[f]	I	5.6 μM	1.7μM[A][g]	PS(2,10)
10712	-	-	-	-	>6 μM	91 μM[A][h]	-
10714	I	I	I	I	>6 μM	ANT[i]	-
10715	I	I	I	I	>6 μM	10 nM(33)	-
10716	I	I	I	I	>6 μM	ANT[j]	NS(1.8-6.4)

a) See text for explanation of column headings and abbreviations.

b) **BIND**: μ=40 nM δ=727 nM, k=86 nM.

c) **PPD**: produced physical dependence. Study terminated because of skin ulcers; **DD**: No discriminative effect; **RF**: non-opioid, similar to competitive NMDA antagonists and ketamine-like drugs; MA: inactive, but augments effect of μ- or k-agonists; **SA**: Maintained rates of responding slightly below alfentanil at one dose only.

d) May exacerbate withdrawal.

e) **PPt-W**: NP(3,12); **RI (PPD)**: low or no physical dependence.

f) Delayed effects or delayed absorption due to vehicle (gum tragacanth).

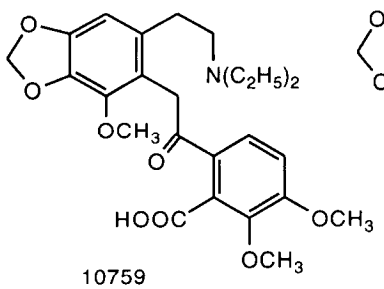
g) Unusual agonist with μ-, δ-, and κ-effects.

h) κ-, or μ-κ agonist actions.

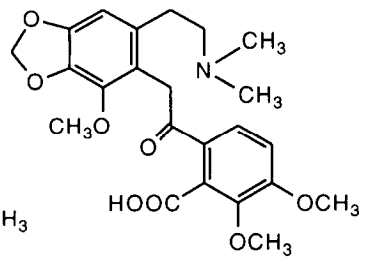
i) μ-, δ-antagonist at high concentrations.

j) Competitive μ-antagonist.

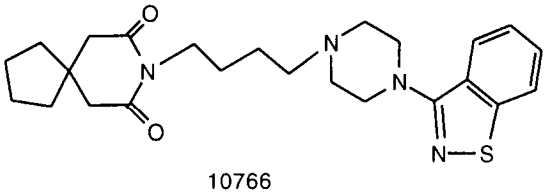
TABLE 9. MISCELLANEOUS (CONTINUED)[a]



10759



10760

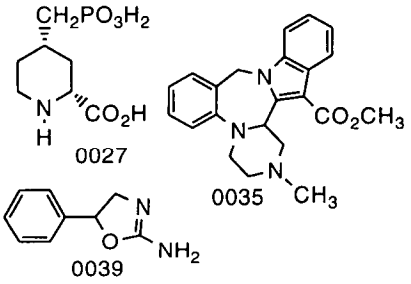


10766

	MOUSE ED50/AD50				IN VITRO		MONKEY
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10759	I	I	I	I	>6 μM	NE	NS(2-24)[b]
10760	I	I	I	I	>6 μM	3.9 nM(41)[c]	NS(2-12)[d]
10766	5.3	0.07	I	I	>6 μM[c]	4.1 μM[c]	PS(0.25,1.0)

a) See text for explanation of column headings and abbreviations.
b) **SA:** Reinforcing properties (like alfentanil) in only one out of four monkeys.
c) Non-opioid or partial low efficacy agonist, u-selective.
d) **SA:** No reinforcing properties.

TABLE 10. EVALUATION OF STIMULANT/DEPRESSANT DRUGS



CPDD#	SLA[a]	IS[b]	PD-S[c]	PD-PPD[d]	SA[e]	DD[f]
0027	DEPRESS.[g]	DEPRESS.	NO[h]	MILD[i]	NO[j]	NO[k]
0035	DEPRESS.[l]	DEPRESS.[l]	NO[m]	-	NO	NO[n]
0039	STIMULANT	NO[o]	YES[p]	-	YES[q]	YES[r]
0040	STIMULANT	NO	-[t]	-	YES[u]	YES[r]

a) Spontaneous locomotor activity (mouse).
b) Inverted screen assay (mouse).
c) Physical dependence - substitution for pentobarbital (rat infusion).
d) Physical dependence - primary (rat infusion).
e) Self-administration (monkey).
f) Drug discrimination (intragastric administration, monkey).
g) Depression: ED_{50}= 3 mg/kg (5 to 8 x more potent than pentobarbital).
h) Did not substitute for pentobarbital to prevent weight loss on withdrawal.
i) Mild abstinence on abrupt withdrawal; unlike pentobarbital or benzodiazepines.
j) Reinforcing effects in one out of three monkeys; ataxic at 1.O mg/kg/inj.
k) Does not share discriminative stimulus effects with c/-amphetamine or pentobarbital after intravenous, intramuscular, or intragastric administration.
l) Slightly more potent than pentobarbital.
m) Lack of suppression may be due low doses necessitated by drug insolubility.
n) Does not share discriminative stimulus effects with &amphetamine or pentobarbital.
o) Impairment observed at highest dose (20 mg/kg) due to toxicity.
p) Exacerbation of withdrawal in pentobarbital-treated rats; potency and duration of action greater than cocaine in cocaine-infused rats.
q) Variability within animals.
r) Discriminative stimulus effects similar to &amphetamine, but not to pentobarbital.
s) Stimulant efficacy and potency slightly greater than cocaine.
t) Insufficient solubility for assay.
u) Reinforcing effects; limited by solubility of compound.

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EVALUATION OF NEW COMPOUNDS FOR OPIOID ACTIVITY ANNUAL REPORT (1993)

J. H. Woods, C. P. France, F. Medzihradsky, C. B. Smith and G. D. Winger

This report contains information on opioid abuse liability evaluations on compounds that have been submitted to the Drug Evaluation Committee of the College and released for publication by the submitters. The information obtained can involve both *in vitro* evaluation in opioid binding assays and smooth muscle (largely, mouse *vas deferens)* preparations. In addition, the compounds may be evaluated for discriminative and reinforcing effects. Analgesic and respiratory function assays are also possible. These behavioral assessments are conducted in rhesus monkeys. Each of these assays is described below. Usually when limited information is provided (*e.g., in vitro* assessment only), it is because the sample provided by the submitter was insufficient to carry out further evaluation.

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, universities, government laboratories, and international organizations are submitted to Dr. Jacobson.

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 Drug Evaluation Committee.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the discriminative stimulus effects of submitted

drugs: one of these groups discriminates the administration of the κ agonist ethylketazocine (EKC); a second group discriminates the μ agonist alfentanil; a third group is treated daily with morphine and discriminates the opioid antagonist naltrexone.

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 cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Bach cycle 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 delivered 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 cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, 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 cycles 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. Bach 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 cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the alfentanil procedure and the EKC procedure is that the alfentanil monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 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 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the alfentanil-trained monkeys are also done using a cumulative dosing procedure with

dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. sessions consist of between two and six discrete, 15-min cycles with each cycle, Under these experimental conditions electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing five times consecutively (*i.e.*, fixed-ratio 5) the lever appropriate for the solution administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug training days a single injection of naltrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (O-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (*e.g.*, irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (*e.g.*, alfentanil; France and Woods, 1989; France et al., 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone (*i.e.*, precipitate withdrawal) are also studied' for their ability to reverse responding on the naltrexone lever in morphine-abstinent (*i.e.*, withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (< 20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

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).

ANALGESIA IN RHESUS MONKEYS

The tail withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40°, 50°, or 55° C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40° C water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40° C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986); however, if a monkey fails to keep its tail in 40° C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40°, 50°, and 55° C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40° C water and less than 5 seconds for 55° C water, monkeys receive the test compound. The test is identical to the pretest, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes or less and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a μ (*e.g.*, alfentanil) or κ (*e.g.*, U-50,488) opioid agonist.

RESPIRATORY FUNCTION STUDIES IN RHESUS MONKEYS

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO_2 in air (France and Woods, 1990; Howell et al., 1988). Monkeys are restrained at the neck and waist while seated in a Plexiglas primate chair. Normal air or 5% CO_2 in air is delivered at a rate of 10 l/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a microprocessor, and are transformed according to known standards to frequency of respiration (f) in breaths/minute and to tidal volume (VT) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to CO_2 The last 3 minutes of exposure to CO_2 are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (*e.g.*, alfentanil).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Bach 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 an intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a 45 sec timeout period occurred. A component of the session ended after 20 injections had been received or 25 min had passed, whichever occurred first. Different doses of the drug was available during each of four components of a session. Other procedural details are given in Winger *et al* (1989).

DISPLACEMENT OF RADIOLABELED LIGAND BINDING

Details of the binding assay based on the displacement of 3H-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. See Table I for representative results with different opioids.

To enhance the characterization of novel opioids, we are also investigating their selectivity in binding to μ-, δ-, and κ-opioid receptors in membranes from monkey brain cortex. Thus, we are now providing EC_{50} 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 (DAMGO); (μ selective),
[D-Pen^{2}-D-Pen^{5}]enkephalin (DPDPE; δ selective),
U-69,593 (κ selective).

Using the receptor-specific assays, we have described the selectivity of various established opioids 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". See Table II for representative results with different opioids in rat and monkey brain.

Based on ligand binding, a method was recently developed for the determination of lipophilicity of opioids (Medzihradsky *et al*., 1992). The procedure offers the routine determination of the octanol-water partition coefficients, requires submilligram amounts of the compounds, and yields

accuracy that is comparable to other, less sensitive and more cumbersome methods of quantitation. Considering the significance of lipophilicity in the function of opioids, the lipid/water partition coefficient should be a valuable biochemical determinant in the preclinical evaluation of opioids (Medzihradsky, 1987).

TABLE I

EC_{50}'s of representative opioids for displacement of 0.5 nM ^{3}H-etorphine from rat brain membrane, and inhibition of the twitch of the mouse vas deferens preparation.

Compound	BINDING* EC_{50} (nM)	MVD
DPDPE	- - -	5.52
U50,488	- - -	6.29
Fentanyl	36.2	37.1
DAMGO	23.9	81.3
Etorphine	0.37	0.0068
(-)Cyclazocine	0.53	11.9
Naltrexone	0.63	- - -
Bremazocine	1.42	0.29
UM 1071R**	1.55	- - -
Sufentanil	1.60	4.43
(-)SKF l0047	3.93	- - -
Ethylketazocine	6.60	11.6
Ketazocine	14.1	1.18
Morphine	23.6	395
DSLET	43.0	1.71
Dextrorphan	<6000	1010

* In the presence of 150 mM NaCl.

**1R-5R-9R-2”R-5,9dimethyl-2’-hydroxy-2-tetrahydrofurfuryl-6obenzomorphanhydrochloride

TABLE II

Inhibition of radiolabeled sufentanil, DPDPE and U69, 593 binding in rat and monkey brain. In membranes from rat cerebrum and monkey brain cortex, the inhibition of specific equilibrium binding of 0.5 nM [^{3}H]sufentil, 1.5 nM [^{3}H]DPDPE and 1.5 nM [^{3}H]U69, 593 by five different concentrations of the listed compounds was investigated in the presence. of 150 mM NaC1 (modified from Clark et al., 1988).

Compound	EC_{50} (nM) [^{3}H]Sufentanil	[^{3}H] DPDPE	[^{3}H]U69,593
Rat Cerebrum			
DAMGO	13.2	690	
Sufentanil	1.25	45.0	
Morphine	31.4	422	
ß-FNA	6.99	43.9	
ß-CNA	1.29	7.48	
Naloxone	6.37	14.3	
Etorphine	0.60	1.13	
Buprenorphine	1.07	1.12	
Bremazocine	1.79	1.12	
Superfit	576	16.5	
DSLET*	121	1.05	
ICI-174,864	58900	59.0	
DPDPE	7720	6.44	
U50,488	7230	13100	
U69,593	38000	13400	
Monkey cortex			
Sufentanil	1.18	81.1	>lOOOO
DPDPE	18900	4.21	>1OOO
U69,593	10700	17000	8.41

*(D-Ser^2,Leu^5)enkephalin-Thr^6

Within our goal to enhance the molecular characterization of novel opioids (Medzihradsky, 1987) we have established functional assays for assessing receptor-effector interactions, reflecting receptor coupling to regulatory G protein and adenylate cyclase, respectively. The methods are

based on the stimulation of brain GTPase and inhibition of adenylate cyclase by opioid agonists, processes blocked by antagonists (Clark and Medzihradsky, 1987; Carter and Medzihradsky, 1992). We are presently evaluating the quantitative responses of partial and irreversible agonists in these assays.

ISOLATED, ELECTRICALLY-STIMULATED MOUSE *VAS DEFERENS* PREPARATION

The development of new, highly selective antagonists such as the reversible K receptor antagonist norbinaltorphimine (Smith *et al.,* 1989) and the competitive δ 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): NaC1, 118; KC1, 4.75; $CaCl_2$ 2.54; $MgSO_4$, 1.19; KH_2 ,PO_4 , 1.19; glucose, 11; $NaHCO_3$, 25; pargyline HCl, 0.3; and disodium edetate, 0.03. The buffer is saturated with 95% O_2 - 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. See Table III for potencies of representative agonists

The following antagonists are studied: naltrexone HC1, ICI-1 74864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and norbinaltorphimine. The antagonists are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. See Table III for the potencies of different competitive antagonists studied in relation to prototypic agonists. EC_{50}'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 a concentration of naltrexone sufficient to block μ,κ and δ receptors. 2) If the submitted drug inhibits the twitch and its actions are blocked by naltrexone, it is evaluated further in the absence and presence of ICI-174864 and norbinaltorphimine used in concentrations at which these antagonists are selective for δ_2 and κ_1 receptors, respectively. 3) If the submitted drug is a partial agonist or devoid of agonistic activity at opioid receptors, it is evaluated further as an antagonist against the following agonists: sufentanil

TABLE III

Potencies of antagonists assessed in the mouse vas deferens

	pA_2 values* determined with three agonists		
	Sufentanil (μ)	U50,488 (κ)	DSLET (δ)
Antagonist			
Naltrexone	8.76	7.74	7.41
Naloxone	7.99	6.90	7.35
Cyprodime*	7.41	6.15	5.98
Nalbuphine	7.23	6.31	5.76
Naltrindole	7.71	7.38	9.44
ICI-174,864	<5.00	<5.00	7.90

*The pA_2 value is the negative logarithm of the molar concentration of antagonist necessary to shift the agonist concentration-effect curve to the right by a factor of 2-fold.

(μ selective), DSLET (δ selective) and U50,488 (k selective). If the submitted drug has antagonistic activity against any or all of the receptor-selective agonists or upon any of the other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive, irreversible) is determined. For further details of the procedure and for a description of experiments in which ß-funaltrexamine was used see Smith (1986). Drugs studied in the preparation prior to 1987 were evaluated with the protocol reported in the 1985 Annual Report.

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 IV. Also shown are dates of Reports to the Biological Coordinator, Dr. A.E. Jacobson, in which results are reported.

TABLE IV
SUMMARY OF TESTS PERFORMED

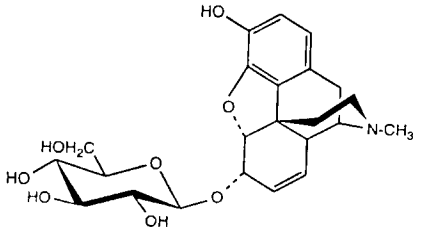
NIH	SA	MVD	BIND	DD	ANLG	RSP	REPORT*
10661	-	+	+	-	-	-	02/08/90
10662	-	+	+	-	-	-	02/08/90
10678	+	+	+ MBC	+	+	+	06/04/91
10707	-	+	+	-	-	-	08/26/91
10708	-	+	+	-	-	-	08/05/91
10711	-	+	+	-	-	-	02/04/92
10712	-	+	+	-	-	-	06/15/91
10714	-	+	+	-	-	-	09/18/91
10715	-	+	+	-	-	-	08/05/91
10716	-	+	+	-	-	-	01/03/92
10735	-	+	+	-	-	-	03/25/92
10738	-	+	+	-	-	-	03/25/92
10739	-	+	+	-	-	-	03/25/92
10740	-	+	+	-	-	-	03/10/92
10741	-	+	+	-	-	-	02/03/92
10742	-	+	+	-	-	-	02/03/92
10743	-	+	+	-	-	-	02/03/92
10744	-	+	+	-	-	-	11/17/92
10745	-	+	+	-	-	-	04/15/92
10748	-	+	+	-	-	-	04/20/92
10749	-	+	+	-	-	-	09/03/92
10750	-	+	+	-	-	-	09/03/92

Table IV (continued)

NIH	SA	MVD	BIND	DD	ANLG	RSP	REPORT*
10751	-	+	+	-	-	-	09/03/92
10572	-	+	+	-	-	-	02/02/93
10753	-	+	+	-	-	-	03/10/92
10754	-	+	+	-	-	-	03/10/92
10755	-	+	+	-	-	-	03/16/92
10756	-	+	+	-	-	-	03/16/92
10757	-	+	+	-	-	-	08/03/92
10759	+	+	+	-	-	-	02/12/93
10760	+	+	+	-	-	-	02/12/93
10761	-	+	+	-	-	-	08/03/92
10763	-	+	+	-	-	-	08/03/92
10766	-	+	+	-	-	-	08/03/92
10768	-	+	+	-	-	-	02/02/92
10769	-	+	+	-	-	-	10/30/92
10770	-	+	+	-	. . -	-	11/12/93
10771	-	+	+	-	-	-	02/09/93
10772	-	+	+	-	-	-	02/09/93
10774	-	+	+	-	-	-	11/12/92
10776	-	+	+	-	-	-	02/12/93
10778	-	+	+	-	-	-	02/12/93
10779	-	+	+	-	-	-	01/19/93
10780	-	+	+	-	-	-	02/12/93
10781	-	+	+	-	-	-	01/19/93
10783	-	+	+	-	-	-	02/12/93
10784	-	+	+	-	-	-	01/26/93
10794	-	+	+	-	-	-	02/09/93
10795	-	+	+	-	-	-	02/15/93
10796	-	+	+	-	-	-	03/05/93

* Date report was submitted to CPDD Biological Coordinator. MBC = Monkey Brain Cortex

NIH 10661 6-O-ß-D-Glucopyranosylmorphine



DISPLACEMENT OF [3H]ETORPHINE BINDING

EC_{50} of 40.4 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

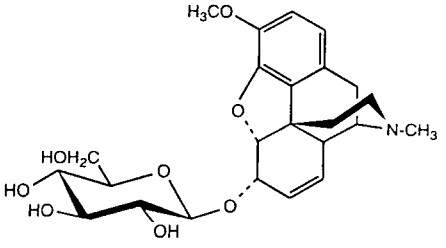
NIH 10661 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. Concentrations between 10^{-8} M and 3 x 10^{5} M caused an inhibition of the twitch. The EC_{50} for NIH 10661 was 1.67 x 10^{-7} M ± 0.26, and the maximum response was a 100% inhibition of the twitch (n= 11). Naltrexone caused a (21.7-fold) shift to the right in the concentration-effect curve. In the presence of naltrexone, 10^{7} M, the EC for NIH 10661 was 3.62 x 10^{-6} M ± 0.29, and the maximum response was a 98.5 ± 1.5% inhibition of the twitch (n=3). Neither ICI-174,864 (a δ antagonist) nor nor-binaltorphimine (a k antagonist) significantly shifted the NIH 10661 concentration-effect curve. In the presence of ICI-174,864, 10^{-7} M, the EC_{50} for NIH 10661 was 4.59 x 10^{-7} M ± 2.45, and the maximum response was a 99.6 ± 0.4% inhibition of the twitch (n=5). In the presence of nor- binaltorphimine, 10^{-8} M, the EC_{50} for NIH 10661 was 3.05 x $1O^{-7}$ M ± 1.15, and the maximum response was a 100% inhibition of the twitch (n=3).

SUMMARY

The *in vitro* preparations indicate significant morphine-like potency. NIH 10661 is selectively antagonized by compounds active at the μ receptor.

* * *

NIH 10662 6-O-ß-D-Glucopyranosylcodeine



DISPLACEMENT OF [^{3}H]ETORPHINE BINDING

EC_{50} of 6377 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10662 was studied upon the isolated, electrically stimulated mouse vas deferens preparation

NIH 10662 (continued)

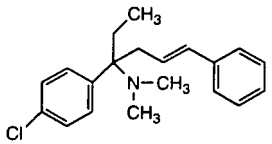
in concentrations which ranged from 10^{-6} M to 3 x 10^{-4} M. Concentrations between 3 x 10^{-6} and 3 x 10^{-4} M caused an inhibition of the twitch. The EC_{50} for NIH 10662 was 1.23 x 10^{-5} M ± 0.25, and the maximum response was a 80.6 ± 9.4% inhibition of the twitch (n=9). Naltrexone and nor-binaltorphimine, but not ICI-174864, blocked the inhibitory actions of NIH 10662. In the presence of naltrexone, 10^{-7} M, the EC_{50} for NIH 10662 was 9.21 x 10^{-5} M ± 1.81 (a 7.5-fold shift), and the maximum response was 70.4 ± 8.8% inhibition of the twitch (n=3). In the presence of nor-binaltorphimine (an antagonist selective for k receptors), 10^{-8} M, the EC_{50} for NIH 10662 was 8.61 x 10^{-6} M ± 7.25, but the maximum response was a 28.9 ± 13.6% inhibition of the twitch (n=3). In the presence of ICI-174864 (an antagonist selective for δ ;receptors), 10^{-7} M, the EC50 for NIH 10662 was 8.86 x 10^{-7} M ±. 4.06, and the maximum response was 53.2 ± 13.3%% inhibition of the twitch (n=3).

SUMMARY

NIH 10662 was a very low potency compound in both preparations. It was a κ agonist in the vas deferens.

* * *

NIH 10678 (-)-1-(4-Chlorophenyl)-N,N-dimethyl-1-ethyl-4-phenylbut-3-en-1ylamine hydrochloride



DISPLACEMENT OF SPECIFIC [³H]ETORPHINE BINDING

EC_{50} of 216 nM in the presence of 150 mM NaCl.

MONKEY BRAIN CORTEX BINDING

This finding was obtained in displacing the specific equilibrium binding of (a) 0.5 nM [³H]DAG0 (μ-selective assay), (b) 1.5 nM [3H]DPDPE (δ-specific assay), and in 1.5 nM [³H]U69,593 (k-selective assay) in membranes from monkey brain cortex suspended in 50 mM Tris.HCl buffer pH 7.4) containing 150 mM NaC1. EC_{50} 's (nM) are as follows:

(a)	μ-receptor	39.6 nM
(b)	δ-receptor	727.0 nM
(c)	κ–receptor	86.2 nM

MOUSE *VAS DEFERENS* PREPARATION

NIH 10678 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-8} M to 3 x 10^{-5} M. Concentrations between 10^{-7} M and 3 x 10^{-5} caused an inhibition of the twitch. The EC_{50} for NIH 10678 was 7.34 x 10^{-7} ± 0.58,

NIH 10678 (continued)

and the maximum response was a 90.3 ± 1.9% inhibition of the twitch (n=9). Naltrexone caused a marked decrease in the magnitude of the maximum response to NIH 10678 without changing the EC_{50} . In the presence of naltrexone, 10^{-7} M, the EC_{50} for NIH 10678 was 6.57 x 10^{-7} M ± 0.46, and the maximum response was a 27.7 ± 7.8% inhibition of the twitch. ICI 174864 (a δ antagonist) did not shift the NIH 10678 concentration-effect curve. In the presence of ICI 174864, 10^{-7} M, the EC for NIH 10678 was 6.62 x 10 M ± 2.00, and the maximum response was a 90.6 ±. 4.4% inhibition of the twitch (n=3). Nor-binaltorphimine (a k antagonist) caused a slight decrease in the maximum response to NIH 10678. In the presence of nor-binaltorphimine, 10^{-8} M, the EC_{50} for NIH 10678 was 7.83 x 10^{-7} M ± 1.13, and the maximum response was a 75.7 ± 4.2% inhibition of the twitch.

DISCRIMINATIVE STIMULUS, ANALGESIC AND RESPIRATORY FUNCTION STUDIES IN RHESUS MONKEYS.

NIH 10678 was studied in rhesus monkeys for its discriminative stimulus effects, analgesic effects, and effects on respiratory function. In monkeys discriminating between saline and 0.0056 mg/kg of the opioid μ agonist alfentanil, NIH 10678 did not substitute completely for alfentanil up to a dose of 3.2 mg/kg. Only on one occasion did subjects respond on the alfentanil-associated lever after receiving 3.2 mg/kg of NIH 10678. This discriminative stimulus effect of NIH 10678 was not clearly antagonized by pretreatment with 0.1 mg/kg of the opioid antagonist quadazocine. The limited solubility of NIH 10678 precludes a complete evaluation of this apparent discriminative stimulus effect.

Up to the largest dose that could be administered (3.2 mg/kg), NIH 10678 failed to substitute for ethylketocyclazocine in normal monkeys and failed to substitute for naltrexone in morphine-treated (3.2 mg/kg/day) monkeys discriminating between 0.032 mg/kg of naltrexone and saline. When saline is substituted for the daily injection of morphine in monkeys discriminating between naltrexone and saline, subjects respond on the naltrexone lever; this naltrexone-appropriate responding is reversed by morphine-like opioids (e.g., alfentanil) and appears to be related to opioid withdrawal. Up to a dose of 3.2 mg/kg, NIH 10678 failed to reverse naltrexone-lever responding in morphine-withdrawn monkeys. Thus, NIH 10678 does not appear to have either μ, k, nor μ antagonist discriminative stimulus effects in rhesus monkeys. NIH 10678 had little or no effect on rates of lever pressing.

NIH 10678 was studied for its effects on the latency of monkeys to remove their tails from warm water. Up to a dose of 3.2 mg/kg, NIH 10678 had no effect on tail withdrawal latency from 50 or 55° C. water. When administered as a pretreatment to a dose-effect determination for alfentanil or the k agonist U-50,488, NIH 10678 appeared to augment the analgesic effects of each agonist as reflected by shifts to the left in the alfentanil and U-50,488 dose-effect curves.

The effects of NIH 10678 on respiratory function were studied in a monkey breathing air or 5 % CO_2 in air. Up to a dose of 0.32 mg/kg, NIH 10678 produced a slight increase in ventilatory frequency (f) and a slight decrease (< 30%) in ventilatory volume (V_T) resulting in little change in minute volume. Doses larger than 0.32 mg/kg decreased V_r further but did not increase f,

producing a net decrease in minute volume. The pattern and magnitude of effects observed with NIH 10678 in studies of respiratory function (decreases in V_T and increases in f) are similar to results obtained in this procedure with nonopioids, including ketamine-like compounds and competitive NMDA antagonists.

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10678 were evaluated in three monkeys experienced in responding and receiving intravenous infusions of alfentanil. The program was one in which four doses of alfentanil were available during each 130 min session. Each dose was available for 25 min or 20 injections and was separated from other dose-availability periods by a 10 min blackout. The schedule of drug delivery was a fixed ratio 30, timeout 45 sec. NIH 10678 was substituted in single test sessions; each test session was separated by at least three sessions in which either alfentanil or saline was delivered contingently on lever press responses.

Doses from 0.0001 to 1.0 mg/kg/inj NIH 10678 were evaluated. NIH 10678 at 0.3 mg/kg/inj maintained rates of responding that were slightly below those maintained by the maximum rate-maintaining dose of alfentanil. No other dose maintained response rates above those maintained by saline.

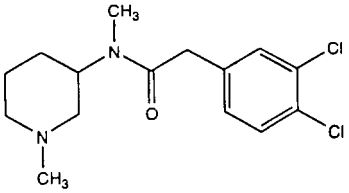
SUMMARY

The *in vitro* data suggest significant opioid activity. The binding data show moderate affinities at μ and κ binding sites. There was some evidence for a partial agonist effect in the *vas deferens* that was sensitive to naltrexone antagonist -- also suggesting μ receptor activity.

The *in vivo* evidence, however, is not compelling for narcotic agonist or partial agonist actions. NIH 10678 failed to produce any discriminative effect indicative of κ or μ activity. It had no activity as an analgesic when given alone, but augmented κ and μ analgesic-induced effects. The compound had modest respiratory depressant effects that, while they resembled excitatory amino acid antagonists (as noted above), did not show the analgesia found with this class of compound. Finally, NIH 10678 was self-injected, a result associated with μ agonists and partial agonists and a variety of nonopioids in this procedure. Thus, NIH 10678 had a novel profile of action.

We have no compounds that have been assessed in humans with which we can make direct comparisons. NIH 10678 is likely, therefore, to produce a set of effects in humans that may be different than μ or κ agonists or partial agonists at these receptors.

NIH 10707 (±)-3-[N-Methyl-N-(3,4-dichlorophenylacetamido)]-N'-methylpiperidine



DISPLACEMENT OF SPECIFIC [³H]ETORPHINE BINDING

EC_{50} of >6,000 nM (7% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

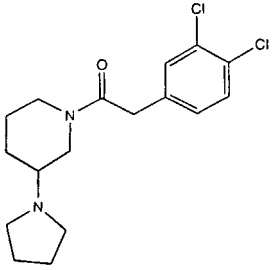
NIH 10707 was devoid of agonist activity on the isolated, electrically stimulated mouse *vas deferens* preparation. This drug, in concentrations up to and including 30 μM, did not block the agonist actions of sufentanil(μ), DSLET (δ) r U50,488 (K). Thus, NIH 10707 does not have opioid activity on the mouse *vas deferens* preparation.

SUMMARY

NIH 10707 had no significant opioid activity in either preparation.

* * *

NIH 10708 1-(3,4-Dichlorophenylacetyl)-3-(1-pyrrolidinyl)piperidine.oxalate



DISPLACEMENT OF SPECIFIC [³]ETORPHINE BINDING

EC_{50} of >6,000 nM (7.4% inhibition at 6 μM) in presence of 150 mM NaCl.

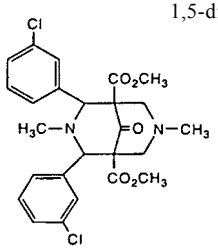
MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift	n
Control	58.0 ± 32.9	21.9 ± 1.2		3
Naltrexone (100 nM)	20.0 ± 3.1	17.4 ± 1.7	0.3	3

SUMMARY

NIH 10708 had no significant opioid activity in either preparation

NIH 10711 4,6-Di-(3-chlorophenyl)-3,7-dimenthyl-3,7-diazabicyclo[3.3.1]nonan-9-one 1,5-dicarboxylic acid



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 5.56 μM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

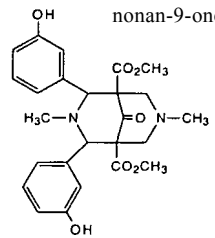
Condition	EC_{50} (μM)	Maximum Response (%)	Shift (x-fold)	n
Control	1.65 ± 0.25	97.8 ± 1.6		9
Naltrexone (100 nM)	10.28 ± 10.12	14.9 ± 4.2	6.2	3
ICI-174864 (100 nM)	5.33 ± 2.29	93.3 ± 6.7	3.2	3
Nor-BNI (10 nM)	5.39 ± 2.25	91.3 ± 5.9	3.3	3

SUMMARY

NIH 10711 was not potent in either assay. It had interesting properties in the *vas deferens* preparation. Its actions were unusual in that, at nearly every concentration between 10 nM and 100 μM, it produced an inhibition of the twitch followed almost immediately by an increase in the magnitude of the twitch. For the purpose of this analysis the response was taken as the greatest inhibition that occurred after administration of each concentration of drug. Naltrexone virtually abolished inhibitory responses to NIH 107 11, but did not affect the increases in twitch magnitude. ICI 174-864, a δ-selective opioid receptor antagonist, and nor-binaltorphimine, a κ-selective opioid receptor antagonist, both shifted the NIH 10711 concentration-effect curve to the right. Thus, NIH 10711 would seem to be an unusual agonist that has actions at μ, κ and δ opioid receptors.

* * *

NIH 10712 4,6-Di-(3-hydroxyphenyl)-3,7-dimethyl-3,7-dimethyl-3,7-diazabicyclo[3.3.1] nonan-9-one 1,5-dicarboxylic acid dimethyl ester



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (39% inhibition at 6 μM) in the presence of 150 mM NaCl.

NIH 10712 (continued)

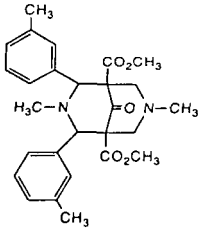
MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (μM)	Maximum Response (%)	Shift	n
Control	0.98 ± 0.01	81.1 ± 3.6		9
Naltrexone (100 nM)	98.0 ± 58.2	100	99.8	3
Nor-BNI (0.1 nM)	1.01 ± 0.21	41.7 ± 4.2	1.0	3
ICI 174854 (100 nM)	1.02 ± 0.05	79.8 ± 3.6	1.0	3

SUMMARY

NIH 10712 was not potent in either preparation. The concentration-effect curve produced by NIH 10712 in the mouse *vas deferens* was shifted to the right by naltrexone and was completely blocked by 10 nM nor-BNI (data not shown). We interpret this pattern of response in the *vas deferens* as a κ agonist action, although a mixed μ-κ agonist action cannot be ruled out.

NIH 10714 3,7-Dimethyl-4,6-di-(3-methylphenyl)-3,7-diazabicyclo[3.3.1]nonan-9-one1,5 dicarboxylic acid dimethyl ether



DISPLACEMENT OF SPECIFIC [^{3}H] ETORPHINE BINDING

EC_{50} of >6000 nM (12% AT 6 μm) in presence of 150mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil	5.84	1.13 ± 0.17	5.88 ± 0.38	6
DSLET	5.50	1.09 ± 0.21	5.50 ± 0.37	6
U50,488				6

Note: Solubility - 3 mM in DMSO. Lack of solubility may affect the reliability of the determined pA2 values.

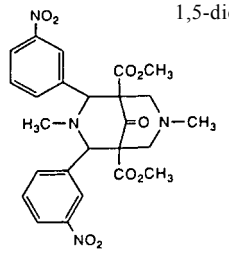
NIH 10714 (continued)

SUMMARY

NIH 10714 was not potent in either assay. At high concentrations, it acted as an antagonist in the *vas deferens* against two of the three agonists. NIH 10714, 10 μM, did not block the action of U50,488. It is not likely to have significant opioid activity at low doses *in vivo*.

* * *

NIH 10715 3,7-Dimethyl-4,6-di(3-nitrophenyl)-3,7-diazabicyclo[3.3.1[nonan-9-one 1,5-dicarboxylic acid



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (10% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

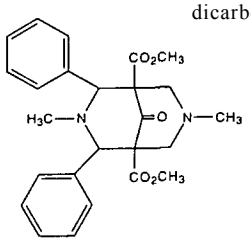
Condition	EC_{50} (nM)	Maximum Response (%)	Shift	n
Control	11.1 ± 3.2	32.8 ± 7.1		3
Naltrexone (100 nM)	7.8 ± 3.7	18.6 ± 1.0	0.7	3

SUMMARY

NIH 10715 was without significant opioid activity in either preparation.

* * *

NIH 10716 3,7-Dimenthyl-4,6-diphenyl-3,7-diazabicycle[3.3.1]nonan-9-one 1,5-dicarboxylic acid dimethyl ester



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (14% inhibition at 6 μM in the presence of 150 mM NaCl.

NIH 10716 (continued)

MOUSE *VAS DEFERENS* PREPARATION

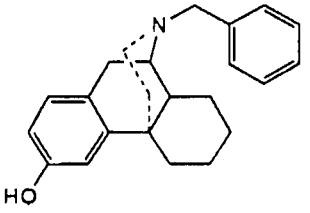
Agonist	pA_2 ± S.E.M.	Slope ± S.E.M.	n
Sufentanil (μ)	8.54 ± 0.33	0.99 ± 0.17	6
U50,488 (κ)	6.40 ± 0.45	1.10 ± 0.20	6
DSLET (δ)	5.84 ± 0.37	1.11 ± 0.14	6

SUMMARY

NIH 10716 was a competitive antagonist in the *vas deferens* with significant selectivity for the μ agonist, sufentanil. It failed to have significant affinity for the etorphine binding site. This is an anomaly.

* * *

NIH 10735 (+)-N-Benzyl-3-hydroxymorphinan hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (32% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10735 was studied on the isolated, electrically stimulated mouse *vas deferens* preparation in concentrations which ranged from 1 nM to 30 μM. Concentrations up to 1 μM had no appreciable effect on this preparation. At concentrations of 3 μM and 10 μM, NIH 10735 markedly increased the magnitude of the twitch. This response was not altered in the presence of 100 nM naltrexone. When tested as an antagonist NIH 10735 (10 μM) did not block the inhibitory actions of sufentanil, a μ opioid receptor agonist. NIH 10735 caused a 3.9-fold shift to the right in the concentration effect curve for DSLET, a δ opioid receptor agonist, and a 14.6-fold shift to the right in the concentration-effect curve for U50,488, a k opioid receptor agonist. Because of the low potency of this drug, pA_2 values were not determined.

NOTE: NIH 10735 was studied in the *vas deferens* at 3 mM in 19% ethanol.

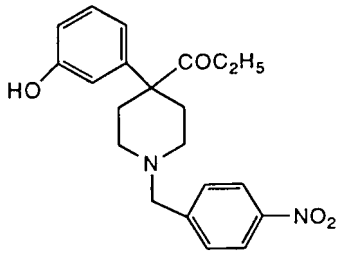
NIH 10735 (continued)

SUMMARY

NIH 10735 was not potent in either preparation. In the *vas deferens* it was devoid of opioid agonist activity, but appeared to be antagonist of very low potency, with some selectivity for **κ** receptors.

* * *

NIH 10738 4-(3-Hydroxyphenyl)-4-ketoethyl-1-(4-nitrobenzyl)piperidinleydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 3.4 μM in the presence of 150 mM NaCl.

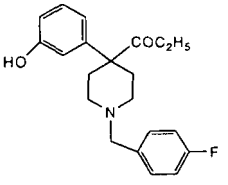
MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	<5.5			3
DSLET (**δ**)	6.18	1.3 ± 0.04	6.38 ± 0.44	6
U50,488 (**κ**)	5.96	1.73 ± 0.42	6.29 ± 0.64	6

SUMMARY

NIH 10738 was of low potency in both preparations. In the *vas deferens* preparation it was devoid of opioid agonist activity. Concentrations of 10 and 30 μM markedly increased the magnitude of the twitch, an action that was not blocked by 100 nM naltrexone. NIH 10738 was an antagonist of low potency on this preparation. It was equipotent as an antagonist at **δ** and **κ** opioid receptors. At a concentration of 10 μM, the compound caused a 9.02-fold shift in the sufentanil concentration-effect curve. Because of its low potency and direct actions on the smooth muscle preparation, pA_2, values could not be calculated for its antagonist activity at μ opioid receptors. The high slopes of the Schild plots suggest that the antagonism produced by NIH 10738 is not a simple competitive type of antagonism.

NIH 10739 1-(4-Fluorobenzyl)-4-(3-hydroxybenzyl)-4-ketoethyl-piperidine hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{5o} of 1330 nM in the presence of NaCl.

MOUSE *VAS DEFERENS* PREPARATION

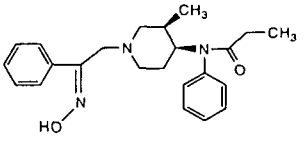
Agonist	pA_2	Slope ± S.D.	pA_2(Constrained ± S.E.	n
Sufentanil (μ)	<5.0			4
DSLET (δ)	6.37	1.66 ± 0.16	6.94 ± 0.59	6
U50,488 (κ)	5.71	1.82 ± 0.52	5.88 ± 0.69	6

SUMMARY

NIH 10739 was not very potent in either preparation. It was devoid of opioid agonist activity on the isolated, electrically stimulated mouse *vas deferens* preparation. Concentrations of 10 and 30 μM markedly increased the magnitude of the twitch, an action which was not blocked by 100 nM naltrexone. NIH 10739 was an antagonist of low potency on this preparation. It was ten times more potent as an antagonist atδ opioid receptors than at k opioid receptors. Although NIH 10739 did not shift the sufentanil concentration-effect curve to the right, it significantly decreased the maximum inhibitory action of sufentanil. NIH 10739, at a concentration of 10 μM, reduced the maximum response to sufentanil to 43.1% of control values. The high slopes of the Schild plots and the changes in the maximum responses to sufentanil suggest that the antagonist produced by NIH 10739 is not competitive.

* * *

NIH 10740 (±)-*cis*-N-[3-Methyl-l-(2-iminohydroxy-2-phenylethyl)-4-piperidinyl]-N-phenylpropanamide (more polar isomer)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 3000 nM in the presence of 150 mM NaCl.

NIH 10740 (continued)

MOUSE *VAS DEFERENS* PREPARATION

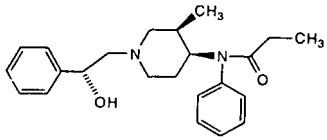
Condition	EC_{50} (μM)	Maximum Response (%)	Shift (x-fold)	n
Control	2.08 ± 0.96	47.1 ± 7.3		8
Naltrexone (100 nM)	0.004 ± 0.001	14.6 ± 14.6	0.0	4
ICI-174,864 (100 nM)	0.58 ± 0.18	24.3 ± 24.3	0.3	4
Nor-BNI (10 nM)	0.31 ± 0.05	46.1 ± 8.6	0.2	3

SUMMARY

NIH 10740 was equally potent in the two preparations. In the mouse *vas deferens* preparation, NIH 10740 was of low potency and efficacy. Not all preparations responded to this drug. Naltrexone, nor-binaltorphimine (a **κ**-selective opioid receptor antagonist) and ICI 174,864 (a **δ**-selective opioid receptor antagonist) had no consistent effects on responses this drug. NIH 10740, in a concentration of 1 μM, did not alter responses to sufentanil (μ-selective agonist), DSLET (**δ**-selective agonist), or U-50,488 {**κ**-selective agonist). Thus, NIH 10740 was devoid of significant opioid activity in the mouse *vas deferens* preparation.

* * *

NIH 10741 (*ßS-3R,4S*)-(+)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride (or (*ßS,3R,4S*)-(+)-*cis-ß*-Hydroxy-3-methylfentanyl hydrochloride)



DISPLACEMENT OF SPECIFIC [³H]ETORPHINE BINDING

$EC_{,,}$ of 5.9 nM in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Higher Affinity Inhibitory Actions

Condition	EC_{50} (femtoM)	Maximum Response (%)	Shift (x-fold)	n
Control	55.9 ± 22.6	100		9
Naltrexone (100 nM)	cannot be calculated	no response		3
ICI-174,864 (100 nM)	70.9 ± 31.2	100	1.3	3
Nor-BNI (10 nM)	76.3 ± 31.2	100	1.4	3

Lower Affinity Inhibitory Actions

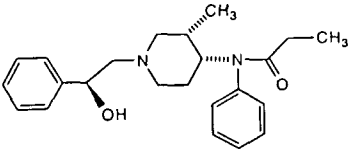
Condition	EC_{50} (mM)	Maximum Response (%)	Shift (x-fold)	n
Control	4.3 ± 22.6	100		9
Naltrexone (100 nM)	21.1 ± 8.0	100	4.9	3
ICI-174,864 (100 nM)	16.9 ± 11.0	100	3.9	3
Nor-BNI (10 nM)	2.2 ± 0.6	100	0.5	3

NIH 10741 was an agonist on the mouse *vas deferens* preparation. It produced an inhibitory action that is associated with a biphasic concentration-effect curve. It was extremely potent with concentrations as low as 3 femtomolar (3 x 10^{15} M) causing an inhibition of the twitch. Naltrexone completely blocked the high affinity actions of NIH 10741. The other antagonists did not alter appreciably the actions of NIH 10741. Both naltrexone and ICI-174864, a δ receptor antagonist, shifted the concentration-effect curve associated with the lower affinity actions of NIH 10741 to the right. Because of the complexity of the actions of this drug, caution should be used in interpreting these results.

SUMMARY

NIH 10741 was quite potent in both assays.

NIH 10742 (*ßR,3S,4R*)-(-)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamidehydroChloride(or(*ßR,3S,4R*)-(-)-*cis-ß*-Hydroxy-3-methylfentanyl hydrochloride)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1023 nM in presence of 150 mM NaCl.

NIH 10742 (continued)

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	140.0 ± 65.9	97.5 ± 1.8		9
Naltrexone (100 nM)	342.2 ± 70.4	98.1 ± 1.9	2.4	3
ICI-174,864 (100 nM)	151.5 ± 68.2	100	1.1	3
Nor-BNI (10 nM)	57.3 ± 29.3	85.4 ± 7.8	0.4	3

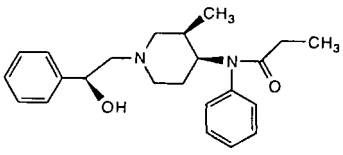
Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained ± S.E.	n
Sufentanil (μ)	6.10	1.42 ± 0.67	6.35	6
DSLET (δ)	5.79	1.50 ± 0.14	6.10 ± 0.67	6
U50,488 (κ)	5.99	2.92 ± 0.91	6.93 ± 1.35	6

SUMMARY

NIH 10742 was less potent than morphine in both assays. On the mouse *vas deferens* preparation, it produced an inhibitory action that is associated with a biphasic concentration-effect curve. It was fairly potent with concentrations as low as 0.03 nanomolar causing an inhibition of the twitch. both phases of the NIH 10742 concentration-effect curve were antagonized by naltrexone and the first phase of the curve was antagonized by both norbinaltorphimine, a κ receptor-selective antagonist, and by ICI-174864, a δ receptor antagonist. It was possible to calculate EC_{50}'s for the second (lower affinity) phase of the concentration-effect curve although these might be somewhat inaccurate because of the complex nature of the control curve. Only naltrexone antagonized the lower affinity actions of NIH 10742.

* * *

NIH 10743 (*βR,3R,4S*)-(-)-*cis*-N-[1-(2(ß)-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide oxalate (or (*βR,3R,4S*)-(-)-*cis*-*β*-Hydroxy-3-methylfentanyl oxalate)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 6.8 nM in the presence of 150 mM NaCl.

NIH 10743 (continued)

MOUSE *VAS DEFERENS* PREPARATION

Higher Affinity Inhibitory Actions

Condition	EC_{50} (femtoM)	Maximum Response (%)	Shift (x-fold)	n
Control	96.4 ± 39.1	53.3 ± 4.3		9
Naltrexone (100 nM)	785.6 ± 378.4	35.4 ± 0.9	8.1	3
ICI-174,864 (100 nM)	194.5 ± 67.5	31.0 ± 2.7	2.0	3
Nor-BNI (10 nM)	510.4 ± 104.2	35.8 ± 0.3	5.3	3

Lower Affinity Inhibitory Actions

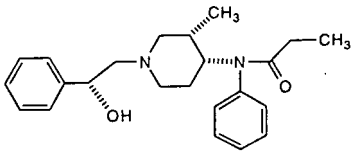
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	6.7 ± 2.8	100		9
Naltrexone (10 nM)	74.5 ± 44.0	100	11.1	3
ICI-174,864 (100 nM)	4.8 ± 2.2	100	0.7	3
Nor-BNI (10 nM)	6.0 ± 2.3	100	0.9	3

NIH 10743 was very similar to NIH 10741 in its actions and in its affinity for opioid receptors. It produced an inhibitory action that is associated with a biphasic concentration-effect curve. It was extremely potent with concentrations as low as 3 femtomolar causing an inhibition of the twitch. This higher affinity action is blocked to some extent by all three receptor-selective antagonists, although naltrexone is much more effective than either ICI-174864 or norbinaltorphimine. The lower affinity actions of NIH 10743 were blocked only by naltrexone, which suggested that this drug was acting at μ opioid receptors. Because of the complexity of the actions of this drug, caution should be exerted in interpreting these results.

SUMMARY

NIH 10743 was a potent opioid in both assays.

NIH 10744 (*βS,3S,4R*)-(+)-*cis*-N-[1-(2(ß)Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]N-phenylpropanamide oxalate
(or (*βS,3S,4R*)(+)-*cis*-*β*-Hydroxy-3-methylfentanyl oxalate)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 380 nM in presence of 150 mM NaC1.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	1.24 ± 0.24	28.1 ± 4.6		9
Naltrexone (100 nM)	6.4 ± 2.8	19.0 ± 0.7	5.1	3
ICI-174865 (100 nM)	2.4 ± 0.6	21.1 ± 5.5	2.0	3
Nor-BNI (10 nM)	4.5 ± 0.7	13.9 ± 2.6	3.7	3

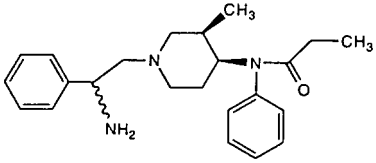
Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.70	0.93	6.62 ± 0.31	3

SUMMARY

NIH 10744 was less potent in the binding than in the *vas deferens* preparation. In the latter, it acted as a weak partial agonist with selectivity for μ opioid receptors. It also was a weak antagonist at μ opioid receptors. It did not block actions of DSLET or U50,488 in concentrations up to 3 μM.

* * *

NIH 10745 (±)-*cis*-N-[1-(2-Amino-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide dihydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 410 nM in the presence of 150 mM NaCl.

NIH 10745 (continued)

MOUSE *VAS DEFERENS* PREPARATION

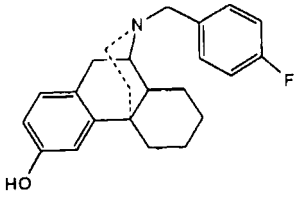
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	76.4 ± 13.5	100		10
Naltrexone (100 nM)	53921.0 ± 5008.2	100	705.8	4
ICI-174865 (100 nM)	55.4 ± 9.1	100	0.7	3
Nor-BNI (10 nM)	118.8 ± 0.0	100	1.6	3

SUMMARY

NIH 10745 had moderate potency in displacing etorphine, and exerted μ-opioid actions in the *vas deferens* preparation.

* * *

NIH 10748 (+)-N-(4-Fluorobenzyl)-3-hydroxymorphinan oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (29% inhibition at 6 μM) in the presence of 150 mM NaCl.

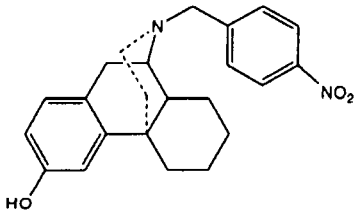
MOUSE *VAS DEFERENS* PREPARATION

NIH 10748 was studied on the isolated, electrically stimulated mouse *vas deferens* preparation in concentrations which ranged from 1 nM to 100 μM. Concentrations up to 1 μM had no appreciable effect on this preparation. Concentrations of 3 μM and greater caused marked increases in the magnitude of the twitch. At a concentration of 300 μM, the twitch was suppressed completely, an effect not blocked by 100 μM of naltrexone. When tested as an antagonist at concentrations up to 30 μM, NIH 10748 did not block the inhibitory actions of sufentanil, a μ opioid receptor-selective agonist, DSLET, a δ opioid receptor agonist, or to U50,488, a κ opioid receptor agonist. Thus, NIH 10748 is devoid of significant opioid agonist activity on the mouse *vas deferent* preparation.

SUMMARY

NIH 10748 was without significant opioid effects in either preparation.

NIH 10749 (+)-3-Hydroxy-N-(4-nitrobenzyl)morphinan oxalate



DISPLACEMENT OF SPECIFIC [³H]ETORPHINE BINDING

EC_{50} of >6000 nM (29% inhibition at 6μM) in presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

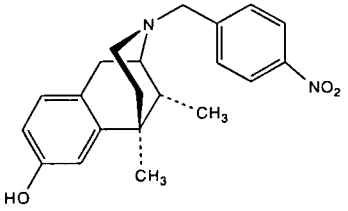
NIH 10749 could not be studied because it was insoluble in the mouse *vas deferens* physiological buffer solution. It was soluble in 48% ethanol at 3 mM, but precipitated when added to Krebs buffer.

SUMMARY

NIH 10749 was without activity in the binding assay.

* * *

NIH 10750 (-)-5,9α-Dimethyl-2'-hydroxy-2-(4-nitrobenzyl)-6,7-benzomorphan oxolate



DISPLACEMENT OF SPECIFIC [³H]ETORPHINE BINDING

EC_{50} of >6,000 nM (16% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	1.84 ± 0.94	37.0 ± 5.1		9
Naltrexone (100 nM)	6.30 ± 4.98	14.1 ± 2.3	3.4	3
ICI-174865 (100 nM)	2.36 ± 0.70	31.3 ± 5.9	1.3	3
Nor-BNI(10 nM)	1.47 ± 0.45	20.1 ± 3.4	0.8	3

NIH 10750 (continued)

Agonist	pA_2	Slope ± S.D.	pA2 (Constrained) ± S.E.	n
Sufentanil (μ)	5.76	1.35	5.84 ± 0.46	3
DSLET (δ)	<5.00			3
U50,488 (κ)	5.70	0.74	5.63 ± 0.25	3

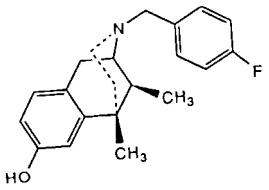
Note: Solubilized at 3mM in 48% ethanol.

SUMMARY

NIH 10750 had no significant activity in the binding assay. In the mouse *vas deferens* preparation, it acted as a partial agonist that was antagonized by naltrexone. It was also a very weak, nonselective antagonist at μ and κ receptors.

* * *

NIH 10751 (+)-5,9 α-Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzo-morphan oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of > 6000 nM (32 % at 6 μM) in the presence of 150 mM NaCl.

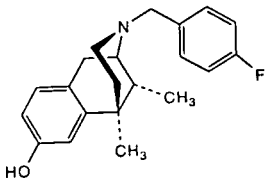
MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	<5.0			3
DSLET (δ)	5.15	1.54	5.23 ± 0.54	3
U50,488 (κ)	6.86	0.51	6.19 ± 0.19	3

SUMMARY

NIH 10751 had no significant activity in the binding assay. It was likewise devoid of opioid agonist activity in the mouse *vas deferens* in concentrations that ranged from 10 nM to 100 μM, at which concentration it increased the magnitude of the twitch. It was a very weak antagonist with some limited selectivity for the κ opioid receptor. The antagonism did not appear to be competitive based upon the slopes of the Schild plots. In concentrations up to 10 μM it did not antagonize the actions of sufentanil, a μ agonist.

NIH 10752 (-)-5,9α-Dimethyl-2-(4-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (27% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

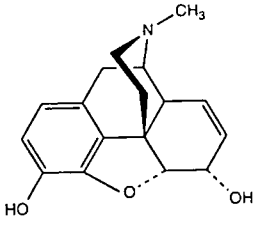
Repeated attempts to dissolve NIH 10752 and to keep in solution when added to physiological solution were unsuccessful. Therefore, EC_{50} values could not be determined.

SUMMARY

NIH 10752 had no significant activity in the binding assay. Data on its effects in the mouse *vas deferens* could not be acquired due to solubility problems.

* * *

NIH 10753 Morphine sulfate pentahydrate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 74.5 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	543.9 ± 38.0	89.4 ± 5.0		11
Naltrexone (100 nM)	1185.0 ± 2669.9	100	20.6	5
ICI-174,864 (100 nM)	965.7 ± 279.5	96.0 ± 4.0	1.8	3
Nor-BNI (10 nM)	716.3 ± 150.6	42.2	1.3	3

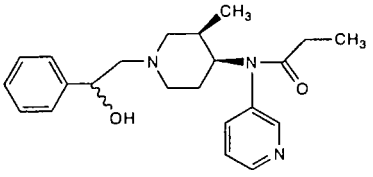
NIH 10753 (continued)

SUMMARY

NIH 10753 was slightly more potent in the binding assay than the *vas deferens.* In the mouse *vas deferens* preparation, it acted as an agonist. Naltrexone and nor-binaltorphimine (a κ-selective opioid receptor antagonist) antagonized the inhibitory actions of NIH 10753. The antagonism produced by nor-binaltorphimine was unsurmountable. ICI-174,864 (aδ-selective opioid receptor antagonist) did not shift the NIH 10753 concentration-effect curve. Thus, in the *vas deferens,* NIH 10753 would seem to be an agonist with actions at μ and K opioid receptors.

* * *

NIH 10754 (±)-*cis*-N-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(3-pyridinyl)propanamide dihydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H] ETORPHINE BINDING

EC_{50} of 28.4 nM in the presence of 150 mM NaCl

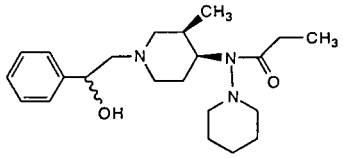
MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	32.1 ± 13.45	100		10
Naltrexone (100 nM)	1431.5 ± 519.3	97.9 ± 2.1	44.6	3
ICI-174,864 (100 nM)	436.6 ± 274.9	100	13.6	4
Nor-BNI (10 nM)	6.97 ± 1.39	100	0.2	3

SUMMARY

NIH 10754 was equally potent in the two preparations. In the mouse *vas deferens* preparation, NIH 10754 acted as an agonist. Naltrexone and ICI 174,864 (a δ-selective opioid receptor antagonist) antagonized the inhibitory actions of NIH 10754. Nor-binaltorphimine, a κ-selective opioid receptor antagonist, did not shift the NIH 10754 concentration-effect curve to the right. Thus, NIH 10754 would seem to be an agonist that has actions at μ and δ opioid receptors.

NIH 10755 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(1-piperidinyl)propanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 377 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

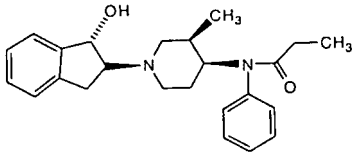
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	89.9 ± 14.1	85.1 ± 5.3		9
Naltrexone (100 nM)	2678.1 ± 2648.7	24.4 ± 8.4	29.8	3
ICI-174,864 (100 nM)	122.6 ± 27.9	87.0 ± 6.6	1.4	3
Nor-BNI (10 nM)	62.3 ± 32.1	89.5 ± 10.5	0.7	3

SUMMARY

NIH 10755 was slightly more potent in the *vas deferens* preparation than in the binding assay. It inhibited the electrically-induced twitch by only 90%; its inhibitory effects were antagonized only by naltrexone, suggesting that its inhibitory effect was completely through the μ receptor.

* * *

NIH 10756 (±)-*cis*-N-[1-(2-trans-l-Hydroxy)indanyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1037 nM in the presence of 150 mM NaCl.

NIH 10756 (continued)

MOUSE *VAS DEFERENS* PREPARATION

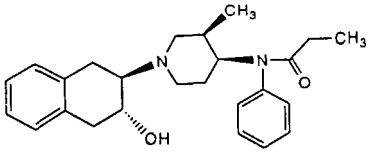
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	309.5 ± 41.7	91.0 ± 3.7		9
Naltrexone (100 nM)	NC	NC		3
ICI-174,864 (100 nM)	443.9 ± 87.4	83.8 ± 7.6	1.4	3
Nor-BNI (10 nM)	315.0 ± 25.3	92.0 ± 2.9	1.0	3

SUMMARY

NIH 10756 was slightly more potent in the *vas deferens* than in the binding assay. On the mouse *vas deferens* preparation NIH 10756 inhibited the electrically induced twitch by 90%. It was markedly antagonized by naltrexone. EC_{50}s could not be determined since, at a concentration of 30 μM and in the presence of 100 nM naltrexone, the response to this drug was negligible; and at a concentration of 100 μ, NIH 10756 completely suppressed the twitch by a nonopioid action. Neither ICI-174864 δ-selective opioid antagonist) nor U-50,488 (κ-selective opioid antagonist) significantly altered responses to this drug. Thus, NIH 10756 would appear to be a partial agonist selective for μ opioid receptors.

* * *

NIH 10757 (±)-*cis*-N-[1-(2-(trans-3-Hydroxy-1,2,3,4-tetrahydro)-naphthyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 48.2 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

NIH 10757 was studied on the isolated, electrically stimulated mouse *vas deferens* preparation in concentrations that ranged from 0.1 nM to 1 μM. Because of the multiphasic nature of the concentration-effect curves, determination of EC_{50}'s was not feasible. NIH 10757 is a fairly potent agonist on the mouse *vas deferens* preparation. Its actions are antagonized by naltrexone and nor-binaltorphimine, but apparently potentiated by ICI 174,864. These results indicate that NIH 10757 acts at opioid receptors, but a definitive classification of the type of receptor(s) that are stimulated is not possible.

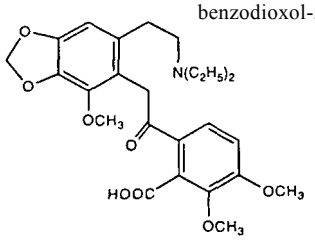
NIH 10757 (continued)

SUMMARY

NIH 10757 had complex actions in the *vas deferens,* and it was comparable to morphine in potency in the binding assay.

* * *

NIH 10759 Ethylnarceine.HCl (6-[[6-[2-Diethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3-dimethoxybenzoic acid



MOUSE *VAS DEFERENS* PREPARATION

NIH 10759 was studied on the isolated, electrically stimulated mouse *vas deferens* preparation in concentrations which ranged from 1 nM to 100 μM. Concentrations up to 1 μM had no appreciable effect on this preparation. Concentrations of 3 μM and greater caused marked increases in the magnitude of the twitch. At a concentration of 300 *μM,* the twitch was suppressed completely, an effect not blocked by 100 nM naltrexone. When tested as an antagonist (at concentrations up to 30 μM) NIH 10759 did not block the inhibitory actions of sufentanil, a μ opioid receptor selective agonist, DSLET, a δ opioid receptor agonist or U50,488, a K receptor selective agonist. Thus, in the *vas deferens* preparation, NIH 10759 was devoid of significant opioid agonist or antagonist activity.

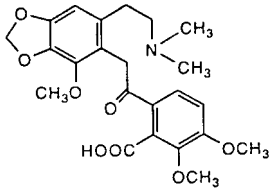
SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10759 were evaluated in four monkeys experienced in responding and receiving intravenous infusions of alfentanil. Doses of from 0.00001 to 3.34 mg/kg/inj were evaluated. One monkey showed high rates of responding maintained by NIH 10759. In this monkey, rates of responding maintained by the maximally effective dose of NIH 10759 (0.32 mg/kg/inj) were as high as those maintained by the maximally effective dose of alfentanil (0.0003 mg/kg/inj). The other three monkeys did not demonstrate a reinforcing effect of 10759. Rates of responding maintained by the drug in these monkeys was usually as low as rates maintained by saline in this situation.

SUMMARY

NIH 10759 had insignificant opioid activity in both the *in vitro* and *in vivo* assays.

NIH 10760 Narceine (6-[[6-[2-Dimethylamino)ethyl]-4-methoxy-1,3-benzodioxol-5-yl]acetyl]-2,3-dimethoxybenzoic acid)



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of > 6,000 nM (2.4 % inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	385.2 ± 108.0	40.5 ± 3.0		9
Naltrexone (100 nM)	821.6 ± 395.8	32.0 ± 8.7	2.1	3
ICI-174,864 (100 nM)	383.5 ± 112.4	29.2 ± 4.4	1.0	3
Nor-BNI (10 nM)	215.8 ± 141.9	39.7 ± 8.1	0.6	3

When evaluated as an antagonist at a concentration of 10 μM, NIH 10760 did not modify responses to sufentanil (μ agonist), DSLET (δ agonist) or U50,488 (κ agonist).

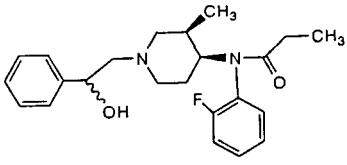
SELF-ADMINISTRATION IN RHESUS MONKEYS

Doses of 0.0001 to 0.3 mg/kg/inj NIH 10760 were evaluated in four monkeys experienced in responding and receiving intravenous infusions of alfentanil. NIH 10760 maintained rates of responding that were well below rates maintained by 0.001 mg/kg/inj alfentanil in all three monkeys. Only one monkey maintained any rates above 0.5 responses per second, the criterion rate when saline is substituted in the procedure.

SUMMARY

NIH 10760 had insignificant opioid activity in the binding assay. In the mouse *vas deferens* preparation, it appeared to be either a non-opioid compound or a partial agonist of very low efficacy with selectivity for μ opioid receptors. NIH 10760 failed to maintain significant self-administration responding.

NIH 10761 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]N-(2-fluorophenyl)propanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1.02 nM in the presence of 150 mM NaC1.

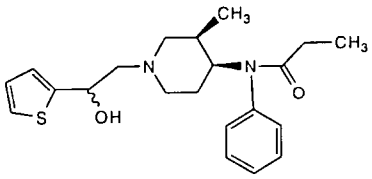
MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	32.5 ± 9.1	98.8 ± 0.7		9
Naltrexone (100 nM)	49.2 ± 1.9	100	1.5	3
ICI-174,864 (100 nM)	67.2 ± 12.6	97.3 ± 2.7	2.1	3
Nor-BNI (10 nM)	61.0 ± 2.3	100	1.9	3

SUMMARY

NIH 10761 acted as an agonist on the mouse *vas deferens* preparation. Its actions were slightly antagonized by naltrexone, ICI-174864 (δ-selective), and nor-binaltorphimine (κ-selective). NIH 10761, at a concentration of 10 nM, caused a 3.1-fold shift to the right in the sufentanil concentration-effect curve and a decrease (31.2%) in the maximum response to this p-receptor-selective agonist. NIH 10761 did not antagonize the actions of U50,488 (κ–selective agonist) or DSLET (δ-selective agonist). Thus, in this assay, NIH 10761 had agonistic effects most consistent with an action at δ opioid receptors and an antagonistic action at μ opioid receptors. NIH 10761 was a potent compound in both preparations. It is unusual that a compound potent in the binding assay and exerts inhibitory actions in the *vas deferens* is also insensitive to the antagonists we employ.

NIH 10763 (±)-*cis*-N-[1-[2-Hydroxy-2-(2-thienyl)ethyl]-3-methyl-4-piperidinyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 5.64 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

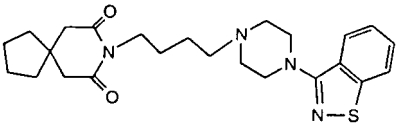
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	113.0 ± 17.7	99.1 ± 0.5		9
Naltrexone (100 nM)	390.0 ± 133.0	100	3.5	3
ICI-174,864 (100 nM)	197.5 ± 59.4	100	1.7	3
Nor-BNI (10 nM)	268.8 ± 146.6	100	2.4	3

SUMMARY

NIH 10763 was an agonist of relatively low potency on the mouse *vas deferens* preparation. Its actions were antagonized slightly by naltrexone and nor-binaltorphimine (κ-selective antagonist), but not by IC-174864 (δ-selective antagonist). NIH 10763, in a concentration of 100 nM, did not antagonize the actions of U50,488 (κ-Selective agonist), sufentanil (μ-selective agonist) or DSLET (δ-selective agonist). Thus, NIH 10763 acted as an agonist in the mouse *vas deferens* prepartion but was devoid of antagonist activity. It was more potent in the binding assay than in the mouse *vas deferens* preparation.

* * *

NIH 10766 8-[4-[4-(1,2-Benzoisothiazol-3-yl)-1-piperazinyl]butyl]-8-azaspiro(4.5)decane-7,9-dione hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of > 6,000 nM (28.2% inhibition at 6 μM) in the presence of 150 mM NaCl.

NIH 10766 (continued)

MOUSE *VAS DEFERENS* PREPARATION

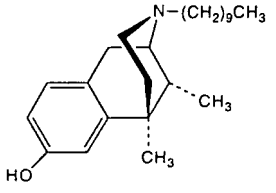
Condition	EC_{50} (μM)	Maximum Response (%)	Shift (x-fold)	n
Control	4.10 ± 0.21	100		9
Naltrexone (100 nM)	5.08 ± 0.72	100	1.2	3
ICI-174,864 (100 nM)	5.69 ± 0.69	100	1.4	3
Nor-BNI (10 nM)	4.71 ± 0.35	100	1.1	3

SUMMARY

NIH 10766 was not potent in either preparation. It is unlikely to have significant opioid actions.

* * *

NIH 10768 (-)-2-Decyl-5,9 α-Dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (43% inhibition at 6 μM) in the presence of 150 mM NaCl.

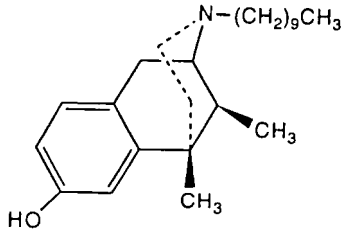
MOUSE *VAS DEFERENS* PREPARATION

NIH 10768 had complex actions on the mouse *vas deferens* preparation. Low concentrations caused an inhibition of the twitch that was antagonized by naltrexone and nor-binaltorphimine, suggesting an action at either μ or κ receptors, or both. Higher concentrations of NIH 10768 caused a further inhibition of the twitch, an action that was not modified by any of the antagonists.

SUMMARY

NIH 10768 was active in the binding assay only a high concentrations. It had weak effects of both opioid and non-opioid character in the *vas deferens* preparation.

NIH 10769 (+)-2-Decyl-5,9 α-Dimethyl-2'-hydroxy-6,7-benzomorphanhydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (26% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

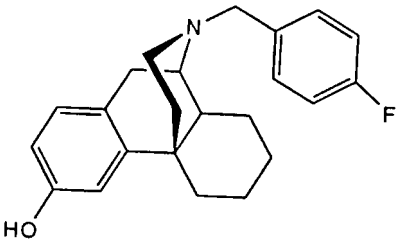
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	13.0 ± 5.6	20.4 ± 2.2		9
Naltrexone (100 nM)	35.5 ± 11.7	14.5 ± 1.8	2.7	3
ICI-174,864 (100 nM)	9.8 ± 4.5	18.1 ± 5.7	0.9	3
Nor-BNI (10 nM)	11.5 ± 3.3	14.9 ± 1.2	0.9	3

SUMMARY

NIH 10769 had insignificant potency in the binding assay. It was either a nonopioid compound or a partial agonist of very low efficacy on the mouse *vas deferens* preparation with selectivity for μ opioid receptors. Concentrations above 0.3 μM increased the magnitude of the twitch. When evaluated as an antagonist at a concentration of 3 μM, NIH 10769 did not modify responses to sufentanil (a μ receptor agonist), DSLET (a δ receptor agonist), or U50,488 (a κ receptor agonist).

* * *

NIH 10770 (-)-N-(4-Fluorobenzyl)-3-hydroxymorphinan oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 1269 nM in the presence of 150 mM NaCl.

NIH 10770 (continued)

MOUSE *VAS DEFERENS* PREPARATION

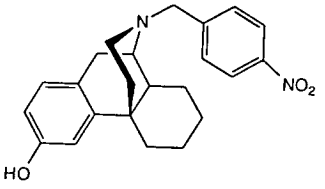
Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	<5.0			3
DSLET (δ)	5.80	0.57	5.68 ± 0.21	3
U50,488 (κ)	6.15	1.31	6.36 ± 0.45	3

SUMMARY

NIH 10770 was of low potency in both preparations. In the *vas deferens* preparation it was devoid of opioid agonist activity in concentrations that ranged from 10 nM to 100 μM at which concentration it increased the magnitude of the twitch. NIH 10770 was a very weak antagonist with some limited selectivity for κ opioid receptors. In concentrations up to 10 μM, it did not antagonize the actions of sufentanil, a μ agonist.

* * *

NIH 10771 (-)-3-Hydroxy-N-(4-nitrobenzyl)morphinan oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 2175 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	7.0 ± 1.6	20.5 ± 2.4		9
Naltrexone (100 nM)	3.4 ± 1.0	14.7 ± 3.1	0.5	3
ICI-174864 (100 nM)	20.2 ± 7.9	11.8 ± 2.3	2.9	3
Nor-BNI (10 nM)	12.7 ± 0.5	9.5 ± 1.1	1.8	3

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.47	0.83	6.40 ± 0.30	3
DSLET (δ)	6.21	0.65	6.21 ± 0.23	3
U50,488 (κ)	6.19	1.56	6.29 ± 0.54	3

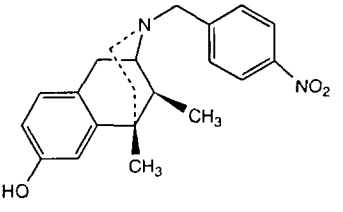
NIH 10771 (continued)

SUMMARY

NIH 10771 had very low potency in the binding assay. In the mouse *vas deferens* preparation it acted as a partial agonist and weak, non-selective antagonist. Although naltrexone did not shift the NIH 10771 concentration-effect curve to the right, it did decrease the maximum response. The other two antagonists shifted the NIH 10771 concentration-effect curve to the right and decreased the maximum response.

* * *

NIH 10772 (+)-5,9 α-Dimethyl-2'-hydroxy-2-(4-nitrobenzyl)-6,7-benzomorphan oxalate



DISPLACEMENT OF SPECIFIC [3H]ETORPHINE BINDING

EC_{50} of >6,000 nM (35% inhibition at 6 μM) in the presence of 150 mM NaCl.

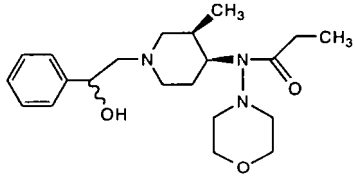
MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.13	0.82	6.02 ± 0.28	3
DSLET (δ)	5.47	1.04	5.49 ± 0.35	3
U50,488 (κ)	5.80	1.61	5.98 ± 0.57	3

SUMMARY

NIH 10772 had no significant activity in the binding assay. In the mouse *vas deferens* preparation it acted as a weak, nonselective antagonist.

NIH 10774 (±)-*cis*-N-[1-2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(4-morpholinyl)propanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 5300 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

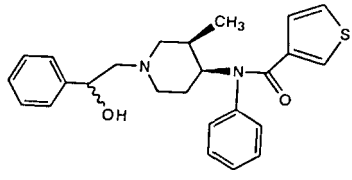
Condition	EC_{50} (μM)	Maximum Response (%)	Shift (x-fold)	n
Control	1.57 ± 0.29	85.1 ± 3.6		9
Naltrexone (100 nM)	12.91 ± 3.14	27.8 ± 7.9	8.2	3
ICI-174,864 (100 nM)	2.36 ± 2.29	55.1 ± 1.9	1.5	3
Nor-BNI (10 nM)	0.92 ± 0.06	79.0 ± 3.9	0.6	3

SUMMARY

NIH 10774 had low potency in both assays. In the *vas deferens,* naltrexone virtually abolished inhibitory responses to NIH 10774. ICI-174,864 (a δ-selective antagonist), caused a slight shift to the right in the NIH 10774 concentration-effect curve and reduced the maximum response. Nor-binaltorphimine (a κ-selective antagonist) neither shifted the concentration-effect curve nor significantly altered the maximum response. When evaluated as an antagonist at a concentration of 1 μM, NIH 10744 did not modify responses to sufentanil (μ-selective agonist), DSLET (δ-selective agonist) or U50,488 (κ-selective agonist). Thus, in the *vas deferens,* NIH 10744 would seem to be an agonist with actions primarily at μ opioid receptors.

* * *

NIH 10776 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-phenyl-3-thiophenecarboxamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 2.83 nM in the presence of 150 mM NaCl.

NIH 10776 (continued)

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	3.42 ± 1.55	100		9
Naltrexone (100 nM)	100.1 ± 12.8	100	29.2	3
ICI-174.864 (100 nM)	17.8 ± 6.7	100	5.2	3
Nor-BNI (10 nM)	2.14 ± 0.56	100	0.6	3

SUMMARY

NIH 10776 had significant opioid activity in the binding assay. It acted as a potent agonist on the mouse *vas deferens* preparation. Both naltrexone and ICI 174,864 (δ antagonist) caused shifts to the right in the NIH 10776 concentration-effect curve. Nor-binaltorphimine (κ antagonist) neither shifted the NIH 10776 concentration-effect curve, nor significantly altered the maximum response. Thus, NIH 10776 would seem to be an agonist with actions at δ opioid receptors -- although an action at μ opioid receptors is also possible. We are currently examining NIH 10776 in binding experiments with receptor-selective ligands.

Note: NIH 10776 was done as a blind control to NIH 10731. Following are the evaluation results from NIH 10731, reported in 1992.

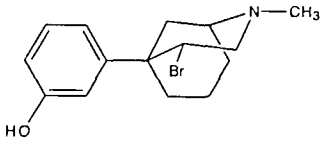
DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 7.3 nM in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	259.2 ± 82.7	67.5 + 3.3		9
Naltrexone (100 nM)	117.3 ± 8.9	98.4 ± 1.6	0.5	3
ICI-174864 (100 nM)	460.0 ± 76.4	36.9 ± 1.3	1.8	3
Nor-BNI (10 nM)	318.2 ± 125.1	51.4 ± 9.9	1.2	3

NIH 10778 4-Bromo-5-(3-hydroxyphenyl)-2-methylmorphan hydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 753 nM in the presence of 150mM NaCl

MOUSE *VAS DEFERENS* PREPARATION

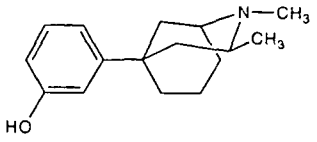
Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.26	0.64	5.82 ± 0.23	3
DSLET (δ)	5.83	1.09	5.90 ± 0.37	3
U50,488 (κ)	5.49	1.17	5.57 ± 0.39	3

SUMMARY

NIH 10778 was of low potency in both preparations. In the *vas deferens* preparation it acted as a weak, non-selective antagonist.

* * *

NIH 10779 2,3-Dimethyl-5-(3-hydroxyphenyl)morphan hydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 592 nM in the presence of 150mM NaCl

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (μM)	Maximum response (%)	Shift (x-fold)	n
Control	15.8 ± 9.8	81.1 ± 7.8		9
Naltrexone (100 nM)	79.7 ± 36.6	75.0 ± 25.0	5.0	3
ICI-174,864 (100) nM)	23.8 ± 18.2	80.8 ± 19.2	1.5	3
Nor-BNI (10 nM)	1.72 ± 1.0	39.6 ± 8.0	0.1	3

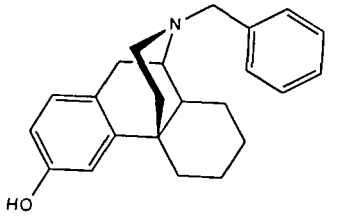
NIH 10779 (continued)

SUMMARY

NIH 10779 had low potency in both assays. It was a partial agonist of low potency on the mouse *vas deferens* preparation with selectivity for μ opioid receptors. When evaluated as an antagonist at a concentration of 1 μM, NIH 10779 did not modify responses to sufentanil, a μ opioid receptor selective agonist, DSLET, a δ opioid-receptor selective agonist, or U50,488, a κ opioid-receptor agonist.

* * *

NIH 10780 (-)-N-Benzyl-3-hydroxymorphinan hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 260 nM in the presence of 150 mM NaCl.

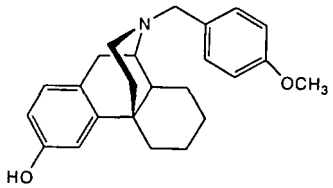
MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.04	0.77	6.03 ± 0.26	3
DSLET (δ)	6.17	0.52	6.10 ± 019	3
U50,488 (κ)	6.49	1.98	6.46 ± 0.78	3

SUMMARY

NIH 10780 was of low potency in both preparations. In the *vas deferens* preparation it acted as a weak, non-selective antagonist.

NIH 10781 (-)-3-Hydroxy-N-(4-methoxybenzyl)morphinan hydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (43% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	5.29 ± 0.91	18.3 ± 3.2		9
Naltrexone (100 nM)	11.9 ± 1.4	18.9 ± 2.4	2.3	3

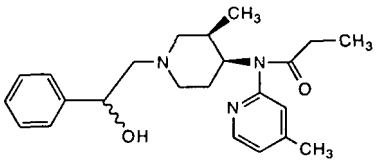
Agonist	pA_2	Slope ± S.E.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	5.66	1.51	5.68 ± 0.37	3
DSLET (δ)	5.69	0.94	5.68 ± 0.16	4
U50,488 (κ)	5.89	2.13	6.33 ± 0.84	3

SUMMARY

NIH 10781 had insignificant affinity for the etorphine binding site. It was a very weak partial agonist that was only slightly antagonized by naltrexone on the isolated, electrically stimulated mouse *vas deferens* preparation. It was also a very weak, nonselective antagonist. The antagonistic actions at μ and κ receptors were probably not competitive as indicated by the slopes of the Schild plots.

* * *

NIH 10783 (+)-cis-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(4-methyl-2-pyridinyl)propanamide oxalate



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 19.1 nM in the presence of 150 mM NaCl.

NIH 10783 (continued)

MOUSE *VAS DEFERENS* PREPARATION

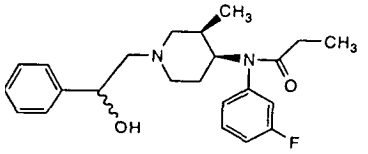
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	7.15 ± 2.12	100		3
Naltrexone (100 nM)	632.7 ± 151.5	100	88.5	3
ICI-174,864 (100 nM)	32.4 ± 27.0	98.6 ± 1.4	4.5	3
Nor-BNI (10 nM)	2.30 ± 0.12	98.6 ± 1.4	0.3	3

SUMMARY

NIH 10783 had opioid activity in the binding assay. In the mouse *vas deferens* preparation, it acted as a potent agonist relatively selective for μ opioid receptors.

* * *

NIH 10784 (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidinyl]-N-(3-fluorophenyl)propanamide hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 6.5 nM in the presence of 150 mM NaCl.

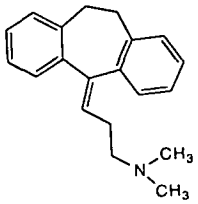
MOUSE *VAS DEFERENS* PREPARATION

Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	7.61 ± 0.26	99.8 ± 0.2		9
Naltrexone (100 nM)	1016.1 ± 208.2	100	133.6	3
ICI-174,864 (100 nM)	126.3 ± 37.4	98.7 ± 1.3	16.6	3
Nor-BNI (10 nM)	109.6 ± 47.8	97.2 ± 1.4	14.4	3

SUMMARY

NIH 10784 had opioid activity in both assays. It was a potent agonist relatively selective for μ opioid receptors on the isolated, electrically-stimulated mouse *vas deferens* preparation.

NIH 10794 Amitriptylene hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (2.4% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

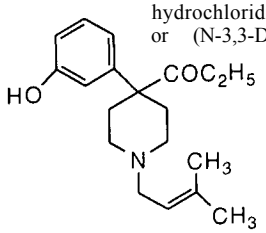
Condition	EC_{50} (nM)	Maximum Response (%)	Shift (x-fold)	n
Control	3.03 ± 1.56	39.4 ± 8.0		3
Naltrexone (100 nM)	17.4 ± 7.0	40.9 ± 3.1	5.7	3

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	5.38	1.71	5.38 ± 0.63	3

SUMMARY

NIH 10794 had no significant activity in the binding assay. In the mouse *vas deferens* preparation it acted as a mixed agonist-antagonist relatively selective for μ opioid receptors. It had low efficacy as an agonist and low potency as an antagonist. Concentration of NIH 10794 above 3 μM markedly increased the magnitude of the twitch. Because of this action and its low potency as an antagonist, pA_2 values could only be determined when sufentanil was used as an agonist.

NIH 10795 4-(3-Hydroxyphenyl-4-(1-oxopropyl)-1-(2-methyl-2-butenyl)piperidine hydrochloride
or (N-3,3-Dimethylallyl)-N-norketobemidone hydrochloride



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of 507 nM in the presence of 150 mM NaCl.

NIH 10795 (continued)

MOUSE *VAS DEFERENS* PREPARATION

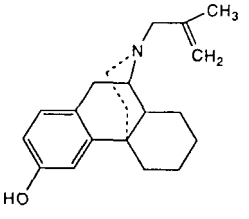
Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	6.58	1.47	6.84 ± 0.52	3
DSLET (δ)	6.06	1.58	6.39 ± 0.56	3

SUMMARY

NIH 10795 had low potency, opioid activity in the binding assay. In the mouse *vas deferens* preparation NIH 10795 produced a slight inhibition of the twitch (26.1% maximum) that was not modified by 100 nM naltrexone. NIH 10795 was a weak, nonselective antagonist on the isolated, electrically-stimulated mouse *vas deferens* preparation. It caused a 23.4-fold shift to the right in the U50,488 concentration-effect curve at a concentration of 30 μM, but pA_2 values were not determined because lower concentrations did not shift the U5O,488 concentration-effect curve. Note that the slopes of the Schild plots are considerably greater than 1.0.

* * *

NIH 10796 (+)-N-(2-Methylpropenyl)-3-hydroxymorphinan hydrobromide



DISPLACEMENT OF SPECIFIC [^{3}H]ETORPHINE BINDING

EC_{50} of >6,000 nM (7.2% inhibition at 6 μM) in the presence of 150 mM NaCl.

MOUSE *VAS DEFERENS* PREPARATION

Agonist	pA_2	Slope ± S.D.	pA_2 (Constrained) ± S.E.	n
Sufentanil (μ)	<5.00			3
DSLET (δ)	<5.00			3
U50,488 (κ)	5.45	1.56	5.68 ± 0.57	3

SUMMARY

NIH 10796 had very low potency in both preparations. In the *vas deferens* preparation it was devoid of agonist activity in concentrations that ranged from 10 nM to 100 μM; at the 100 μM concentration, it increased the magnitude of the twitch. It was a very weak antagonist with some selectivity for κ opioid receptors. In concentrations up to 10 μM it did not antagonize the actions of sufentanil or DSLET.

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PROGRESS REPORT FROM THE TESTING PROGRAM FOR STIMULANT AND DEPRESSANT DRUGS (1993)

G. A. Patrick, L. S. Harris, W. L. Woolverton, M. A. Nader, G. Winger and J. H. Woods

The research group involved in the evaluation of stimulant and depressant compounds has been in existence for approximately ten years. The group includes laboratories at Virginia Commonwealth University (Patrick, Harris), the University of Chicago (Wootverton, Nader), the University of Michigan (Winger, Woods) and NIH (Jacobson). The group is part of the Drug Evaluation Committee, chaired by Ted Cicero, of the College on Problems of Drug Dependence (CPDD) and is supported by both CPDD and NIDA. One of the purposes of the group is to evaluate new compounds, generally classified as either stimulants or depressants, for their abuse liability and potential to produce dependence. Compounds are received, coded and distributed by Dr. Jacobson for blind testing in the various laboratories. They are evaluated for discriminative stimulus effects (UC), reinforcing effects (UM), and capacity to produce physical dependence (VCU). This report includes the results of evaluation of the following compounds: CPDD-0027 ([cis-4-phosphomethyl]-2-piperidinecarboxylic acid); CPDD-0035 (1.3.4.16b-tetrahydro-2-methyl-2H,10H-indolo[2,1-c]pyrazino[1,2a][1,4]benzodiazepine-16-carboxylic acid methyl ester hydrochloride or CGS-15044A); CPDD-0039 (aminorex hydrochloride); and CPDD-0040 (mesocarb).

METHODS

Reinforcing Effects in Rhesus Monkeys

The reinforcing effects of test compounds were evaluated in a substitution self-administration procedure with methohexital serving as the baseline drug. Rhesus monkeys were surgically prepared with indwelling silicone rubber catheters using 10mg/kg i.m. ketamine and 2.0 mg/kg i.m. xylazine as aesthetics. Catheters were implanted in jugular (internal or external), femoral or brachial veins as necessary. The catheter passed subcutaneously from the site of the incision to the mid-scapular region, where it exited the monkey and continued, through a hollow restraining arm, to the outside rear of the cage.

The restraint and catheter protection device has been described in detail by Deneau *et al.* (1969). Monkeys were individually housed in stainless steel cages, measuring 83.3 X 76.2 X 91.4 cm deep. Each monkey wore a tubular stainless steel harness that protected the exit site of the catheter and allowed relatively unrestricted movements within the cage. A Teflon cloth jacket (Alice King Chatharn Medical Arts, Los Angeles, CA) provided further protection for animals who tended to locate and pull their catheters. The harness was connected to a flexible spring arm that carried the catheter to the back of the cage where it joined tubing passing through a roller infusion pump (Watson and Marlow Co., Model MHRK 55, Falmouth, UK).

A 15.4 cm square stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 2.5 cm apart, were three circles, 2.5 cm in diameter, covered with translucent plastic and capable of being illuminated from behind by 5 W colored bulbs. The two side lights could be illuminated ted and the center light green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD) capable of being operated bv 10-15 gm of force. Experimental control was provided by an IBM PS/2 computer programmed with Med-PC (Med-Associates, Fairfield, VT) software and located in an adjoining room.

Monkeys were adapted to restraining arms for a week or more, then an intravenous catheter was implanted and the animals were given the opportunity to respond to receive drug. Evaluation of drugs with depressant properties was carried out in monkeys trained to self-administer sodium methohexital. For these monkeys, at the beginning of each session, a red light was illuminated over one of two levers in each monkey's cage and 10 responses (fixed-ratio 10; FR10) on that lever resulted in a 5-second infusion of 0.1 mg/kg sodium methohexital, followed by a 10-second time-out during which all stimulus lights were extinguished and responding had no programmed consequence. During an infusion, the red lever light was extinguished and the center green light illuminated for the duration of the infusion. Experimental sessions were limited to 210 min or until a maximum of 200 injections were delivered. No monkey ever received 200 injections of methohexital. Two sessions were scheduled each day, separated by at least four hours. On approximately half of the baseline sessions, the monkeys were exposed to response-contingent saline. When them was a clear and consistent differential response between saline and methohexital, a dose of the test compound was substituted for one session. All conditions were similar to training sessions except the maximum number of injections of the test compound was limited to 150/session. Each dose was tested twice in each monkey.

CPDD-0027,0035 and 0039 were dissolved in distilled water; CPDD-0040 was dissolved in a vehicle of equal parts Emulphor EL-620 and ethanol (each $\leq$ 10 % of total volume) diluted with distilled water.

Discriminative Stimulus Effects in Rhesus Monkeys

The subjects were two female and five male rhesus monkeys weighing between 6.5 and 12.1 kg. All monkeys had extensive experience with the present drug discrimination procedure. They were housed individually in stainless steel cages in which water was continuously available. They were fed 100 to 150 g of monkey chow after each session and were given a chewable vitamin tablet 3 days/week. During experimental sessions each monkey was seated in a Plas-Lab restraining chair and placed in a wooden cubicle (175 cm high X 85 cm wide X 65 cm deep) containing two response levers mounted 100 cm above the floor. A 40 W white house light was mounted on the ceiling. The monkey's feet were placed into shoes, the bottoms of which were fitted with brass plates which could deliver electric shocks. Programming and recording of experimental events were accomplished by an Aim 65 microprocessor located in an adjacent room.

The monkeys had been trained previously to discriminate d-amphetamine (AMPH: 7737, 7739.8515) or pentobarbital (PB: 8106, 8236, 7976, 8814) from saline in a two-lever, discrete-trial shock avoidance procedure similar to the one descibed by Holtzrnan (1982). One hour after an intragastric infusion (via nasogastric tube) of the training drug (0.56 or 1.0 mg/kg AMPH or 10 mg/kg PB) or saline, the houselights and lever lights were illuminated (trial) and responding on one lever (designated the correct lever) avoided electric shock and extinguished the lights. Responding on the incorrect lever started a 2-second change-over delay during which correct lever responding had no consequence. If a correct lever response was not made within 5 seconds of onset of the lights, an electric shock (250 msec duration, 7mA intensity) was delivered; if a correct response was made within 2 sec after the first shock (escape), the trial was terminated, otherwise., a second shock automatically ended a trial. Two consecutive trials with escape failure automatically ended the session. Trials were separated by a 30-sec TO. The session lasted 30 trials or 20 min, whichever came first The correct lever was determined by the infusion that was administered before the session. For three monkeys, the right lever was correct after drug infusion and the left lever was correct after saline infusions. This condition was reversed for the other four monkeys.

Monkeys were considered to be stable in the discrimination when more than 90% of the trials were completed on the correct lever on at least seven out of eight consecutive sessions. At this point, testing was begun with the training drugs and the test drugs. Two

5-day sequences alternated drug, vehicle and test sessions so that the first test session was preceded by two training sessions, one with saline and one with drug pretreatment and the second test session of the sequence was preceded by either vehicle or drug pretreatment. In the event that the criterion for stimulus control was not met during the training sessions, the training sequence was continued. During test sessions, both levers were operational, *i.e.,* shock could be avoided by responding on either lever.

Saline, at least three doses of the training drug, and three doses of each test drug, in addition to the test drug vehicle, were evaluated under the test conditions for each monkey. The percentage of trials that were completed on the drug lever is presented for each test session. In addition, the average time between the onset of a trial and a lever press (average latency) was calculated for each test session. Because these test compounds were evaluated blind without any dose-response information, initial test doses were done in an ascending or&r from 0.1 mg/kg to doses that either significantly increased latency to respond or resulted in at least 90% drug-appropriate responding. Out of concern for the monkeys, doses greater than 30 mg/kg were not tested. If a dose substituted for a training drug, that dose and doses higher and lower were tested again, in a random order.

PB and the test drugs were prepared immediately before testing, while a stock solution of AMPH was prepared each week. PB (40 mg/ml) and AMPH (5 mg/ml) were dissolved in saline. CPDD-0027, 0035, and 0039 were dissolved in water. CPDD-40 was dissolved in ethanol (10%), propylene glycol (40%) in saline.

Physical Dependence Studies in Rats and Potency Estimation in Mice

Male Sprague-Dawley rats (Harlan Labs, Dublin, VA) initially weighing 200-225 g were individually housed in stainless steel cages with food and water freely available. They were used in the chronic infusion and substitution experiments. CF-1 mice (Harlan Labs, Dublin, VA) weighing 25 to 35 g were housed in plastic cages with food and water *ad lib.* The mice were used in initial studies for potency estimation. All animals were acclimated to the animal facility for several days prior to use in any study.

Rats were surgically prepared with an intraperitoneal cannula (PE90 tubing) while under methoxyflurane anesthesia Acclimation to the infusion system occurred for three days during which the rats were infused with 0.9% saline. This was followed by the continuous infusion of either saline (control) or pentobarbital sodium for 12 consecutive days using an escalating dosing schedule (Yutrzenka *et al.,* 1985). At the end of the infusion period most rats were receiving pentobarbital at a dose of 850-900 mg/kg-24 hr. Body weight was monitored daily during the drug infusion period.

Following the final day of pentobarbital infusion, a 24-hour substitution period commenced during which pentobarbital-dependent rats were infused with either saline, vehicle, or test drug. This was followed by a 24-hour drug withdrawal period during which all rats received saline.

Every two hours for the first 12 hours and again at 24 hours of each period, rats were assigned a withdrawal score based on the degree of expression of several behavioral responses and signs. In addition, body weight was determined at 0.8 and 24 hours of each period. Scores were assigned by two observers who were blind to the drug treatment. Investigators were blind to the identity of the compounds until all data were collected and analyzed (Yutrzenka *et al.,* 1989).

In the primary physical dependence study with CPDD-0027, rata were infused with a solution of the test drug continually for 12 days, using an escalating dosage schedule. Following cessation of the drug infusion, saline was substituted for 48 hours, and overt behavioral signs of withdrawal and changes in body weight were measured as described above.

Preliminary studies to ascertain potency of the test compounds were conducted in mice. Drug-treated mice were assayed using the inverted screen test (Coughenour *et al.,* 1977) and alteration of spontaneous locomotor activity. At least three doses of each drug, with at least six mice per dose, were used to determine dose-response curves. Vehicle-treated mice served as controls and were assayed concurrently with drug-treated mice.

The inverted screen test was conducted at 20, 30, 60, and 120 min following drug administration. The ED-50 dose, which was determined to be the dose at which one-half of the treated mice failed to right themselves within the 60-second time period, was computed for each time period where appropriate. Spontaneous locomotor activity was determined using a single beam photocell (Autotron, Inc., Danville, IL,) which bisected a plastic cage containing two mice (CPDD-0027, 0035 and 0039) or using a 16-beam infrared photocell (Omnitech, Columbus, OH) which transected a plastic cage containing one mouse (CPDD-0040). Movement of the mice disrupted the beam(s) and a "count" of activity was recorded. Following drug administration, activity was recorded at 5-15 min, 35-50 min, 65-95 min, and 125-185 min. The ED-50 dose for a depressant drug was determined to be that dose which reduced spontaneous locomotor activity to one-half that recorded for concurrently tested vehicle-treated control mice. For stimulant drugs, activity at a given dose was expressed as "percent of control activity". Potency estimates of each test drug were determined at time of peak activity and when, in addition, the vehicle effect was no longer evident.

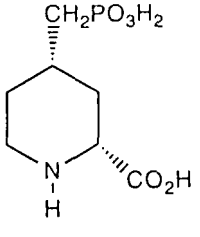
Pentobarbital sodium was dissolved in distilled water made isotonic with sodium chloride. CPDD-0027 and 0039 were dissolved in distilled water. CPDD-0035 was dissolved in propylene glycol (40%) and ethanol (10%) in distilled water, and 0040 was dissolved in Emulphor EL-620 (10%) and ethanol (10%) in distilled water.

Withdrawal scores for each treatment group were compared to the control by use of the Mann-Whitney U-test. Alterations in body weight were tested for significance by use of the t-test. ED-50 values and 95% confidence intervals in the inverted screen test and locomotor activity measure were also determined when appropriate by the method of Litchfield and Wilcoxon (1949).

RESULTS

CPDD-0027

(cis-4-phosphomethyl)-2-piperidinecarboxylic acid



Self-administration Studies in Rhesus Monkeys

The reinforcing effects of doses of 0.1 through 1.0 mg/kg/injection of compound 0027 were evaluated in three rhesus monkeys. All doses maintained fairly low drug intake (number of injections) relative to the number of injections of methohexital taken. However, number of injections taken of CPDD-0027 tended to increase as the dose of drug increased (*see* Figure 1). Larger doses could not be evaluated due to solubility problems. At the largest dose tested (1.0 mg/kg/inj) the number of injections taken of 0027 was above those maintained by saline in one of two monkeys. The third monkey was not evaluated at this dose. Two of the three monkeys showed direct effects of the drug administration after the session. One

showed ketamine-like effects (e.g. salivation, apparent anesthesia with eyes open). The other showed decreased rates of methohexital-maintained responding of the subsequent session.

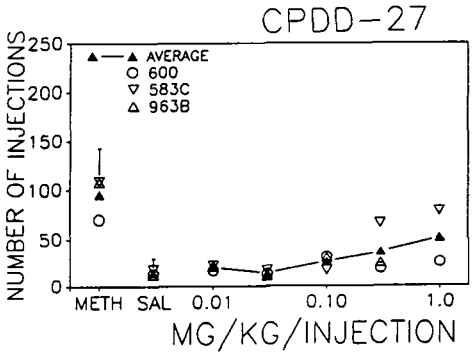


Figure 1. Self-administration of CPDD-0027 by rhesus monkeys. The points shown at METH are the average number of injections taken of 0.1 mg/kg/inj sodium methohexital on the sessions just preceding each substitution of CPDD-0027. These data are shown for the individual monkeys as indicated by the legend. Similar data are shown for saline availability over the point indicated by SAL. Data shown for CPDD-0027 are averaged across at least two observations for each monkey.

Drug Discrimination Studies in Rhesus Monkeys

When given i.g., CPDD-0027 occasioned only saline-appropriate responding in all monkeys tested in doses from 0.1 to 30 mg/kg. There were no systematic effects of i.g. 0027 on response latency. Parenteral administration of CPDD-0027 occasioned primarily saline-lever responding in all monkeys, although some PB-appropriate responding was observed (see Table 1). One PB-trained monkey (8106) made 100% drug-lever responding following 10 mg/kg (i.v.) CPDD-0027; however, when this dose was retested, only saline-lever responding resulted; and a higher dose eliminated responding. Parenteral administration of CPDD-0027 also increased latency to respond relative to saline controls and changes in overt behavior were noticed (e.g., two monkeys would not leave their home cages at the start of the session). These results demonstrate that CPDD-0027, administered intragastrically or parenterally, does not have discriminative stimulus effects similar to those of PB or AMPH in rhesus monkeys.

Table 1

Effects of parenteral administration of CPDD 0027 in PB- or AMPH-trained monkeys

Subject	Training Drug	Saline	1.0	3.0	5.6	10	17	30
Pentobarbital-trained								
8236	100/0.37	0/0.1				0/0.1	1.7/0.03	31.5/0.27
7976	100/0.99	0/0.02				0/0.50	0/0.07	38/0.83*
8106	100/0.20	0/0.1		0/0.07		50.3/0.0	**	
8814	92/0.53	0/0.40		0/0.47		0/0.17	15.5/0.99*	
d-Amphetamine-trained								
7739	100/0.96	0/0.60	0/0.72	0/0.57	0.0/0.72	***		
8515	100/0.25	0/0.1		33.5/0.05		6.7/0.10	0/0.13	0/0.63
7737	93.5/0.95	0/0.33	0/0.57	0/0.69		6.5/0.67	0/0.47	***

Rhesus monkeys were trained to discriminate either 10 mg/kg (i.g.) pentobarbital or 0.56-1.0 mg/kg (i.g.) d-amphetamine from saline in a discrete-trials avoidance/escape paradigm. Data presented represent the percent drug-appropriate responding/average response latency (sec). Generally, CPDD 0027 was administered intravenously 60 minutes prior to testing, except for 7739 who received CPDD 0027 intramuscularly 4 hrs prior to testing. Except where noted, 30 trials were completed.

* shocks were received and less than 30 trials were completed

** no responses made during the session

*** drug was administered but monkey could not be placed in the chair.

Potency Estimation in Mice

CPDD-0027 produced a dose-related depression of activity most evident from 35 to 95 min after treatment (see Table 2), with an ED-50 of approximately 3 mg/kg (making it about 8 times as potent as pentobarbital). This effect gradually abated between 2 and 3 hours after treatment. Performance on the inverted screen task was affected from 20 to 120 min post-treatment, with an ED-50 of approximately 5 mg/kg. This effect was largely dissipated by 4 hours after drug administration.

Table 2. Effects of CPDD Compound #0027 on Spontaneous Locomotor Activity

	Time after treatment (min)			
Dose (mg/kg)	5-15	35-50	65-95	125-185
1.25	99[a]	99	58	77
2.5	79	50	70	248
5	58	17	20	47
10	55	8	18	105

[a]Value expressed as percent of control activity of concomitantly tested, vehicle-treated mice.

****

Physical Dependence Studies in Rats

Substitution and Withdrawal in Pentobarbital-dependent Rats

On the basis of the potency estimates gleaned from the study of acute effects on performance in the inverted screen task and on locomotor activity, doses of CPDD-0027, equal to one-fifth (180 mg/kg-24 hr) and one-tenth (90 mg/kg-24 hr) of the final dosage of PB, were infused into rats that had been chronically infused with PB.

Substitution of CPDD-0027 significantly affected the overt behavioral signs of withdrawal from PB only at 8, 10 and 24 hours into withdrawal (see Figure 2a). When 0027 was discontinued and saline was substituted, there was no significant elevation of signs suggestive of withdrawal from the compound (Figure 2b). These results suggest that CPDD-0027 substitutes only mildly for PB in dependent animals.

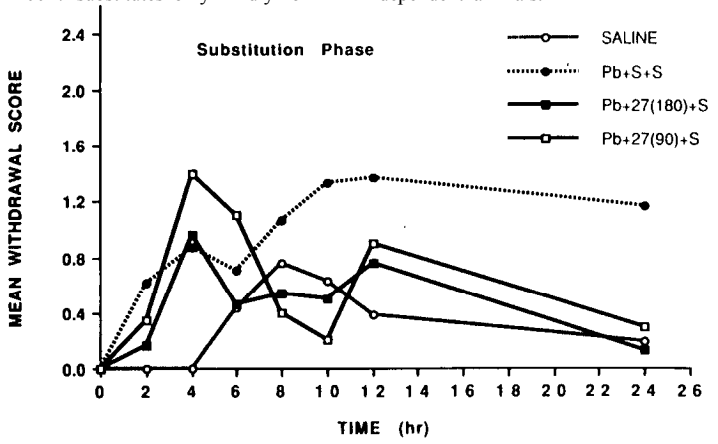


Figure 2a. Meann withdrawal score of control rats and PB-dependent rats during substitution of CPDD-0027 or vehicle. CPDD-0027 was infused in doses of 90 and 180 mg/kg-24 hr. (n=3 to 6)

Figure 2b: Withdrawal Scores on Substltutlon of Saline(S) and CPDD#0027

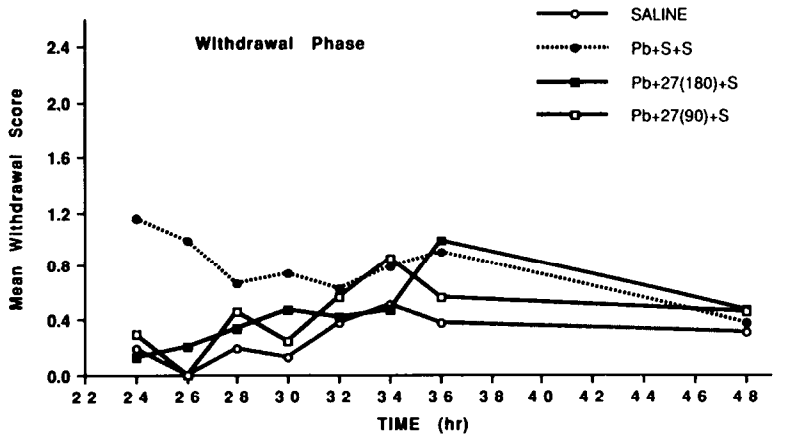


Figure 2b. Mean withdrawal score of control and P-Bdependent rats during substitution of saline (24 to 48 hr into withdrawal, the "Withdrawal Phase"). (n=3 to 6)

Changes in body weights of animals during substitution of CPDD-0027 and during withdrawal were more conclusive. As shown in Figure 3, rats receiving CPDD-0027 suffered an even greater loss of weight than vehicle-substituted rats, and the pattern of changes in weight was the same for vehicle- and 0027-substituted animals. Some of the rats receiving CPDD-0027 appeared sedated during the substitution phase of the experiment, but the sedation did not appear to be sufficient to suppress feeding.

Figure 3: Changes In Body Weight with Substitution of Saline(S) and CPDD#0027

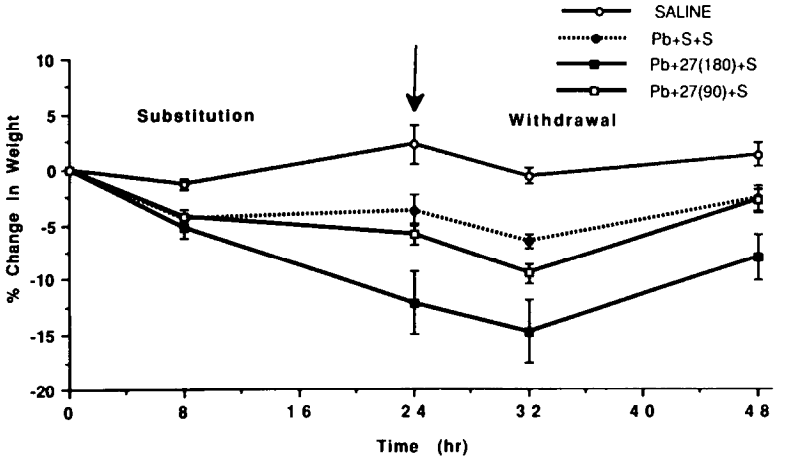


Figure 3. Change in body weight of control and PB-dependent rats during substitution of vehicle or CPDD-0027 (0 to 24 hr. substitution phase) and substitution of saline (24 to 48 hr, withdrawal phase). CPDD-0027 was infused in doses of 90 and 180 mg/kg-24 hr. (n=3 to 6)

Primary Physical Dependence Liability in Rats

During infusion of CPDD-0027 the rats became quite sedated and hypoactive with diminished muscle tone. One rat died of apparent overdose on the eleventh day of infusion. During the course of infusion, rats gained little or no weight, in contrast to the frequent 20 to 30 % weight gain seen in rats infused with PB for the same period. Dosage began at 20 mg/kg-24 hr on the first day of infusion and concluded at 200 mg/kg-24 hr for most rats on the twelfth day of infusion.

When saline was substituted for CPDD-0027, there was little observance of overt signs of abstinence, particularly for the first 12 hours (see Figures 4a & 4b). During the 24 to 36 hour period, there was a mild but significant elevation in signs, approximating those seen in PB-abstinent rata during the same period. Changes in body weight during substitution of saline did not reflect a barbiturate-like withdrawal pattern (see Figure 5). In fact, rather than exhibiting the weight loss that is characteristic of barbiturate abstinence, the CPDD-0027-withdrawn rats gamed weight relative to saline-infused control animals.

FIGURE 4A: WITHDRAWAL SCORES ON SUBSTITUTION OF SALINE(S) AND CPDD #0027

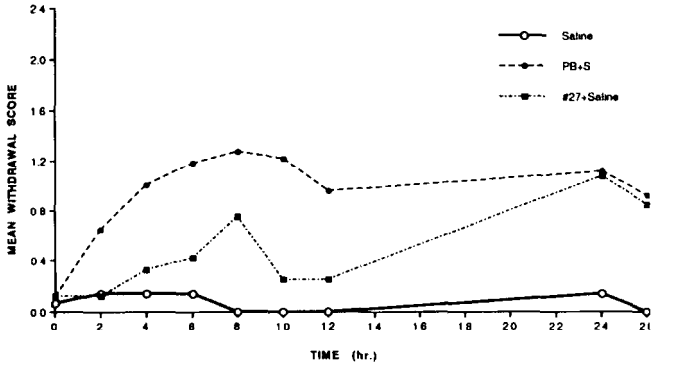
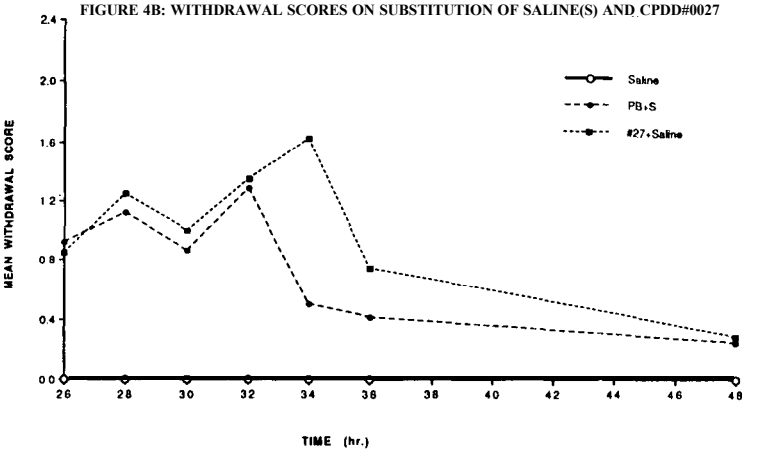


FIGURE 4B: WITHDRAWAL SCORES ON SUBSTITUTION OF SALINE(S) AND CPDD#0027



Figures 4a & 4b. Mean withdrawal scores of control rats, PB-dependent rats, and CPDD 0027-infused rats during substitution of saline (0 to 48 hr). CPDD 0027 was infused in a final dose of 200 mg/kg-24 hr. (n=4 to 6)

FIGURE 5: CHANGE IN BODY WEIGHT WITH SUBSTITUTION OF SALINE IN RATS INFUSED WITH PB OR CPDD #0027

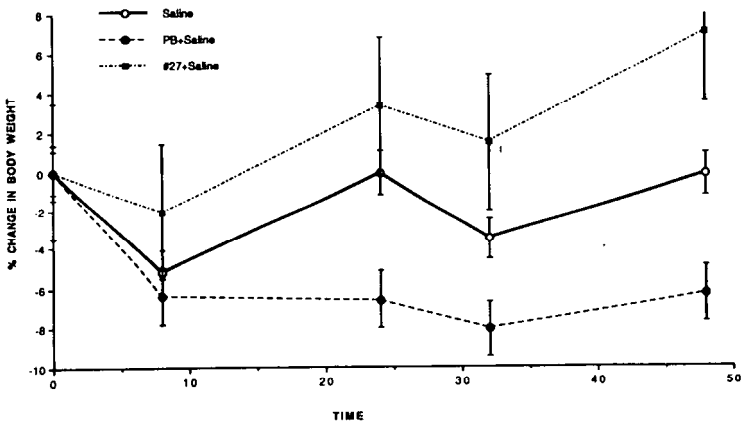
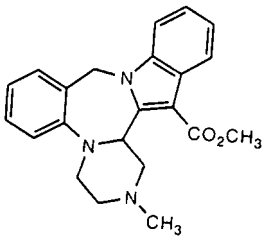


Figure 5. Change in body weight of control rats, PB-dependent rats, and CPDD 0027-infused rats during substitution of saline (0 to 48 hr). (n = 4 to 6)

CPDD-0035

1,3,4,16b-Tetrahydro-2-methyl-2H,10H-indolo(2,l-c)pyrazino(1,2-a)(1,4)benzodiazepine-16-carboxylic acid methyl ester hydrochloride or CGS-15044A



Self-administration Studies in Rhesus Monkeys

CPDD-0035 did not maintain self-administration in any of the three rhesus monkeys evaluated. Doses of from 0.001 to 0.1 mg/kg/inj maintained injection levels similar to those maintained by saline.

Drug Discrimination Studies in Rhesus Monkeys

Administration of CPDD-0035 occasioned primarily saline-appropriate responding in all monkeys. With the exception of monkey 7737, doses of 0035 up to 30 mg/kg i.g. did not substantially affect average response latency or overall activity of the monkey in the homecage. Monkey 7737 would not leave his homecage 55 min after receiving 30 mg/kg of CPDD-0035.

Potency Estimation in Mice

CPDD-0035 produced a dose-related depression of spontaneous locomotor activity up to 60 min after treatment, with an ED-50 at the 5 to 15 min interval of 11.3 mg/kg (3.9 - 32.3 mg/kg, 95% confidence limits) and an ED-50 at the 35 to 50 min interval of 13.3 mg/kg (5.6 -31.4). The effect abated rather dramatically over the next 2 hours.

Performance on the inverted screen task was affected rather erratically, with a dose-responsive effect observed only at the earliest time of measurement (20 min). The ED-50 at that time was 25.2 mg/kg (0.75 - 840). This effect was dissipated largely by 30 to 60 min after drug administration, with only occasional animals affected at 60 min and later.

These data suggest that CPDD-0035 is only slightly more potent than PB in these tests of depression, being roughly equipotent in the inverted screen test and less than twice as potent in the suppression of locomotor activity.

Physical Dependence (Substitution) Study in PB-dependent Rats

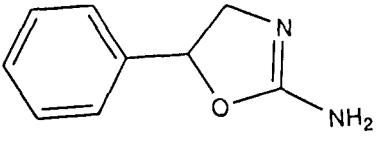
CPDD-0035 was infused into PB-dependent rats at doses of 100 and 200 mg/kg-24 hr (n = 3 to 7 per group). These doses are substantially below equipotency with the final dose of PB received, but the limited solubility of the drug precluded administration of higher doses.

When CPDD-0035 was substituted for PB, there was no significant effect on overt signs of abstinence. Likewise, when the compound was discontinued and saline was substituted for it, there was no significant increase in signs of abstinence.

Changes in body weight during withdrawal also failed to show suppression of abstinence by CPDD-0035. The pattern of weight loss was similar in the drug-substituted and vehicle-substituted groups of rats.

CPDD-0039

Aminorcx hydrochloride (4,5-Dihydro-5-phenyl-2-oxazolamine hydrochloride)



Self-administration Studies in Rhesus Monkeys

Monkeys allowed to self-administer compound 0039 in doses of from 0.001 to 0.1 mg/kg/inj self-administered nearly as many injections of this drug as they did of the baseline dose of methohexital. Maximum number of injections were taken at a dose of 0.03 mg/kg/inj (see Figure 6).

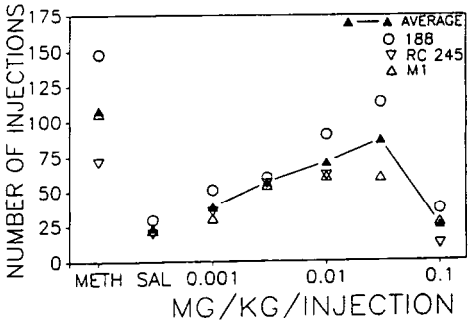


Figure 6. Self-administration of CPDD-0039 by rhesus monkeys. The points shown at METH are the average number of injections taken of 0.1 ms/kg/inj sodium methohexital on the sessions just preceding each substitution of CPDD-0039. These averages are shown for the individual monkeys as indicated by the legend. Similar data are shown for saline availability over the point indicated by SAL. Data shown for CPDD-0039 are averaged across at least two observations for each monkey.

Drug Discrimination Studies in Rhesus Monkeys

CPDD-0039 produced a dose-related increase in drug-lever responding in all three AMPH-trained monkeys. Full substitution for AMPH was found with at least one dose in all three monkeys (see Figure 7a). Latency to respond was not systematically affected (Figure 7b).

Fig. 7a

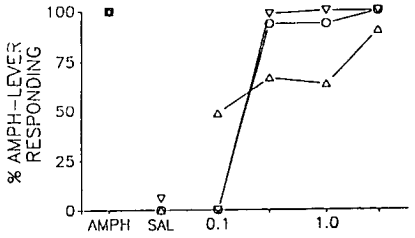


Fig. 7b

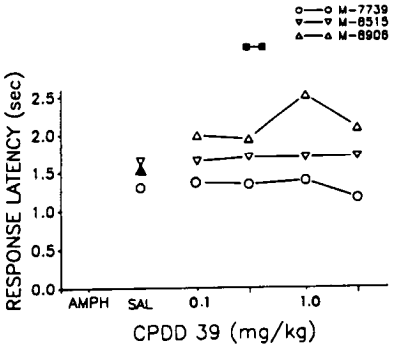


Figure. 7. (a) Discriminative stimulus effects of CPDD-0039 in rhesus monkeys trained to discriminate amphetamine from saline. Each point represents the percentage of trials in a test session that were completed on the drug lever. Generally, each point is the mean of two determinations and this is always the case for points that are above 80%. (b) Latency to respond in the same trials.

Potency Estimation in Mice

CPDD-0039 caused a mild increase in locomotor activity at 0.625 mg/kg, and the stimulant effect became significant at 1.25 mg/kg. Stimulation was dose-related between 1.25 and 20 mg/kg, but not markedly so (see Table 3). The highest dose tested (20 mg/kg) exhibited significant toxicity in that it was the only dose that caused impairment of performance in the inverted screen test (from 58% impairment at 20 min post-treatment declining to 20% impairment at 120 min). That dose was lethal in one of six mice.

Table 3: Effects of CPDD Compound #0039 on Spontaneous Lacomotor Activity

	Time after injection (min)			
Dose of #39	5-15	35-50	65-95	125-185
0.625	149a	124	196	234
1.25	180	238	488	82
2.50	270	183	674	152
5.00	244	379	180	176
10.00	303	460	1074	
20.00	95	132	562	3010

a Values are expressed as the percentage of activity of concomitantly tested vehicle-treated control mice.

Physical Dependence (Substitution) Studies in Chronically-infused Rats

PDD-0039 was infused i.p. in doses of 40 and 80 mg/kg-day for 24 hr into rats that had been infused with pentobarbital (PB) for 12 days. There were 4 to 6 rats in each treatment group.

When CPDD-0039 was infused into PB-dependent rats, the overt signs of withdrawal were markedly increased (see Figure 8). In addition, tremor was noted in many of the rats receiving the compound. The exacerbation of withdrawal symptomatology was dose-related, and the elevation of signs persisted for 24 hr following cessation of the higher dose. The weight loss associated with barbiturate abstinence was also significantly increased by substitution of compound 0039 (Figure 9), although there was no difference in the degree of effect between the two doses.

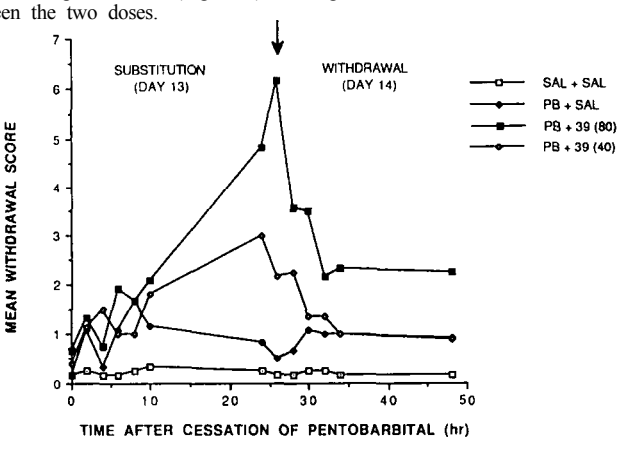


Figure 8. Mean withdrawal scores of control rats and PB-dependent rats during substitution of CPDD-0039 or vehicle (Day 13, Substitution) and during substitution of saline (Day 14, Withdrawal) (n=4 to 6)

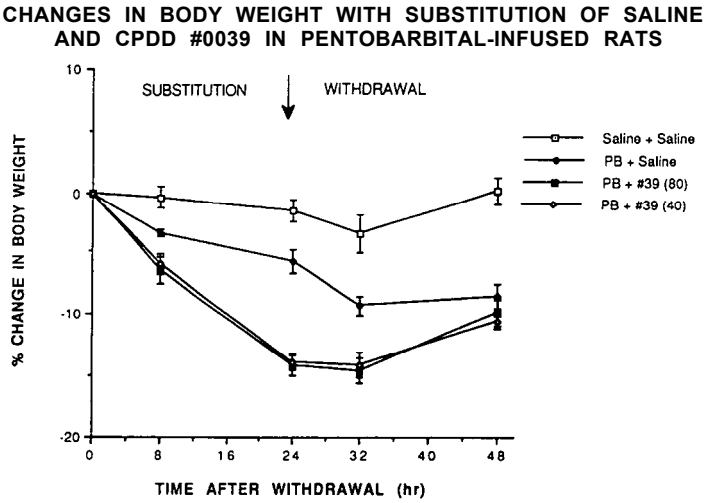
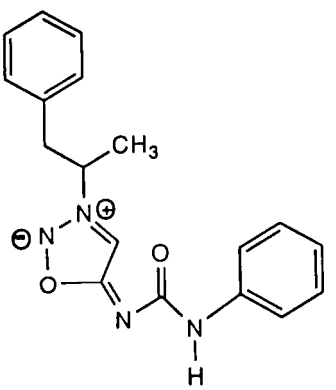


Figure 9. Change in body weight of control rats and PB-dependent rats during substitution of CPDD-0039 or vehicle (0 to 24 hr, Substitution) and during substitution of saline (24 to 48 hr, Withdrawal) (n = 4 to 6)

CPDD-0040

Mesocarb (3-[1-methyl-2-phenethyl]-N-[phenylaminocarbonyl]sydnone imine)



Self-administration Studies in Rhesus Monkeys

CPDD-0040 produced a dose-related increase in rates of drug-maintained responding over a dose range of 0.003 to 0.03 mg/kg/inj (see Figure 10). At a larger dose of 0.1 mg/kg/inj, rates of responding were somewhat decreased. A reinforcing effect of 0040 was observed in each of the three monkeys in which it was evaluated.

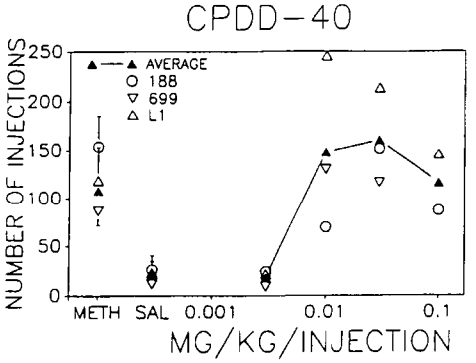


Figure 10. Self-administration of CPDD-0040 by rhesus monkeys. The points shown at METH are the average number of injections taken of 0.1 mg/kg/inj sodium methohexital on the sessions just preceding each substitution of CPDD-0040. These averages are shown for the individual monkeys as indicated by the legend. Similar data are shown for saline availability over the point indicated by SAL. Data shown for CPDD-0040 are averaged across at least two observations for each monkey.

Drug Discrimination Studies in Rhesus Monkeys

CPDD-0040 did not occasion pentobarbital-lever responding in any monkey tested up to doses of 1.0 mg/kg. Compound 0040 did produce a dose-related increase in AMPH-lever responding in AMPH-trained monkeys. Full substitution for AMPH was found with at least one dose in all monkeys (see Figure 11). Latency to respond was not systematically affected.

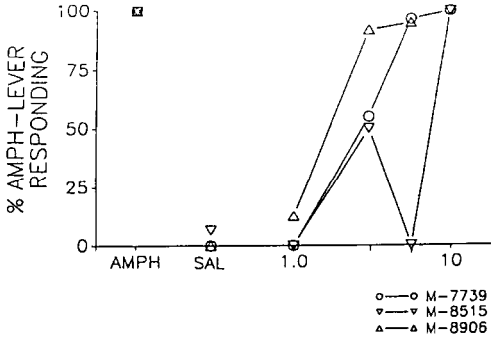


Figure 11. Discriminative stimulus effects of CPDD-0040 in rhesus monkeys trained to discriminate amphetamine (AMPH) from saline. Each point represents the percentage of trials in a test session that were completed on the drug lever. Generally, each point is the mean of two detcrminations and this is always the case for points that are above 80%.

Potency Estimation in Mice

CPDD-0040 caused a mild increase in activity at 1.25 mg/kg, and the effect became significant at 2.5 mg/kg. The stimulant effect was dose-related, although the increase in activity with each increment in dosage was rather mild (see Table 4). No dose tested exhibited significant toxicity nor caused impairment of performance in the inverted screen test. Inability to solubilize the compound sufficiently has precluded evaluation of dependence liability.

Table 4: Effects of CPDD Compound #0040 on Spontaneous Locomotor Activity

Dose of #40 (mg/kg)	Time after injection (min) 5-15	35-50	65-95	12 5-185
1.25	160	222	142	184
2.50	110	210	227	425
5.0	137	255	298	424
10.0	280	407	385	745
20.0	192	316	431	1020

a Values are expressed as the percentage of activity of concomitantly tested vehicle-treated control mice.

CONCLUSIONS

CPDD-0027, an NMDA antagonist, did not have reinforcing effects in rhesus monkeys, and it possessed only mild pentobarbital-like discriminative stimulus effects in the same species. In mice and rats. it produces definite sedative effects. CPDD-0027 produced occasional mild suppression of overt behavioral signs of withdrawal in pentobarbital-dependent rats, and mild behavioral signs were exhibited when rats were withdrawn from it after chronic infusion. However: it did not suppress the weight loss associated with barbiturate withdrawal, nor did rats withdrawn from it lose weight but rather gained weight. Although this compound does exhibit behavioral effects typical of CNS depressant drugs, it appears unlikely to possess barbiturate-like abuse liability or the potential-to produce physical-dependence characteristic of the barbiturates or benzodiazepines.

CPDD-0035 exhibited neither reinforcing properties nor barbiturate-discriminative stimulus properties in rhesus monkeys. In mice it produced short-lived depressant effects. At doses that could be solubilized, this compound did not affect the course of withdrawal in pentobarbital-dependent rats. Therefore, this compound seems unlikely to possess significant potential for abuse or for promoting physical dependence.

CPDD-0039 (Aminorex) exhibited marked reinforcing properties in rhesus monkeys. It also produced dose-related drug discrimination in amphetamine-trained monkeys, fully substituting for amphetamine at some dosage in all animals tested. The compound produced acute stimulant effects in mice, and it exacerbated withdrawal when infused into pentobarbital-dependent rats. These results indicate that CPDD-0039 should have reinforcing effects and abuse liability similar to amphetamine and other potent psychomotor stimulants.

CPDD-0040 (Mesocarb) produced reinforcing effects in self-administration studies in rhesus monkeys. Similarly, it produced amphetamine-like discriminative stimulus effects, although it was less potent than CPDD-0039 in this regard. It also produced acute stimulant effects in mice. This compound, like aminorex, appears to have reinforcing properties and abuse liability resembling the psychomotor stimulants.

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