
Preface to the Report:

The Advantages and Limitations of Calmatives for Use as a Non-Lethal Technique

The Applied Research Laboratory has been helping U.S. law enforcement and military agencies develop an information base on which to make decisions about minimal force options for conflict resolution since 1997. The premise of minimal force option studies, often referred to as non-lethal or less-than lethal “weapons,” is whether there are appropriate alternatives to lethal force in order to minimize the loss of life.

Given the recent increase in global terrorism and our own experiences of September 11, 2001, the need exists for effective and safe techniques that can deal with belligerent individuals who exploit innocent bystanders for concealment or hold them hostage. Our aim is to provide a scientific basis for understanding the options being contemplated.

Societal benefits and ethical concerns need to be addressed. Reasoned opinions (*New York Times* editorial 10/30/02) have been expressed that in “an age of terrorism, it would surely be desirable to develop a mist that could put people to sleep quickly without harming them permanently.” Other voices have been raised in disagreement.

It is our view that the pursuit of minimal force options and the debate surrounding them should be conducted on the basis of existing facts from the scientific literature. Policy and legal aspects of developing and employing such technology should certainly be considered as well.

The following literature/library search was prepared in that context as an internally funded initiative and basis for discussion. The literature search was provided to the National Academy of Sciences’s Naval Studies Board and considered in their expert panel assessment of non-lethal alternative technologies.

PENNSSTATE



College of Medicine
Applied Research Laboratory
The Pennsylvania State University

The Advantages and Limitations of Calmatives for Use as a Non-Lethal Technique

Dr. Joan M. Lakoski
Dr. W. Bosseau Murray
Dr. John M. Kenny

October 3, 2000

Contents

EXECUTIVE SUMMARY	2
INTRODUCTION	5
The Research Objective	5
The Subject of Review	5
Contribution of the Report on Calmatives	6
Important Observations from Literature Search on Calmatives	6
CALMATIVES – ADVANTAGES AND LIMITATIONS	7
What is a Calmative?	7
Pharmacological Effects of a Calmative	7
Preclinical and Clinical Information on Calmatives	8
An Ideal Non-Lethal Calmative	9
Required Use of Medical Attention with States of Unconsciousness	10
Specific Advantages of Calmatives Over Other Non-Lethal Techniques	10
LITERATURE SURVEY – METHODOLOGY	12
The Search Process	12
Profile of the Calmative Database	12
SELECTED CALMATIVES – RESULTS	15
Rationale for Topics Selected	15
Benzodiazepines	16
Alpha ₂ Adrenergic Receptor Agonists	20
Dopamine D3 Receptor Agonists	21
Selective Serotonin Reuptake Inhibitors	26
Serotonin 5-HT _{1A} Receptor Agonists	28
Opioid Receptors and Mu Agonists	32
Neurolept Anesthetics	36
Neurolept Anesthetic Combinations	38
Corticotropin-releasing Factor Receptor Antagonists	39
Cholecystokinin B Receptor Antagonists	42
RECOMMENDATIONS	46
Continuing Improvements in Drug Delivery	46
New Improvements with Combinations of Drugs	47
Developing Partnerships with the Pharmaceutical Industry	48
Closing Comments	48

Executive Summary

The purpose of this study was to assess the potential use of calmatives as non-lethal techniques. This research included 1) defining the advantages and limitations of pharmaceutical agents as calmatives with potential use as non-lethal techniques, 2) providing a comprehensive survey of the medical literature identifying pharmaceutical agents that produce a calm state and developing this information into a database of the relevant literature on calmatives, 3) providing an in-depth review of selected calmatives identified by the literature search with high potential for further consideration as a non-lethal technique, and 4) to identify and provide recommendations on new areas in pharmaceutical drug development that may uniquely meet the requirements of calmatives as non-lethal techniques.

Sidebar text highlights salient points throughout the report.

There may be a need for development of non-lethal techniques with a high degree of specificity, selectivity, safety and reversibility that would avoid production of a lasting impairment to the subject(s) or individual(s) activating the technique. Pharmaceutical agents, or calmatives, with a profile of producing a calm-like behavioral state were considered highly appropriate for consideration in the design, enhancement, and implementation of non-lethal techniques. While ethical issues are involved with the use of calmatives in this context, consideration of these issues was beyond the scope of this project. The Researchers concentrated on the task of defining the ideal characteristics of a non-lethal calmative technique, conducting extensive literature searches and providing an in-depth analysis of this material. In addition, the Researchers have provided recommendations for the development and use of calmatives and other pharmaceutical agents, including convulsants, as non-lethal techniques.

Pharmaceutical agents considered under the topic of “calmatives” include compounds known to depress or inhibit the function of the central nervous system. Several major classes of pharmacological compounds under this category include sedative-hypnotic agents, anesthetic agents, skeletal muscle relaxants, opioid analgesics, anxiolytics, antipsychotics, antidepressants and selected drugs of abuse. Drugs which depress the nervous system have a range of effects that are dependent on the dose and duration of drug administered; these physiological and behavioral effects range from amelioration of anxiety, mild sedation, hypnotic effects to coma and death. Pharmaceutical compounds recommended for use as non-lethal calmatives will typically not be administered to produce deep sedation or hypnosis; rather, calmatives will be used to relieve anxiety and produce mild sedation. Moreover, the compounds featured for in-depth discussion in this report, have unique characteristics that offer specific advantages in a non-lethal warfare setting.

This report highlights the pharmacological effects of a calmativ e, including a discussion of pharmacokinetic and pharmacodynamic principles of drug action in the central nervous system. The pharmacological effects include consideration of a calmativ e's route(s) of administration, rate of absorption and distribution, biotransformation and excretion profiles, mechanism of actions, as well as consideration of known side effects. The importance of data on calmativ es obtained from both preclinical and clinical research was considered to be vitally needed information in the assessment of a calmativ e agent. Additional consideration was also given to research with calmativ es conducted in patient populations with a range of disruptive behaviors, ranging from treatment of withdrawal from alcohol, alleviation of debilitating anxiety concomitant with social phobia, therapeutic treatment of violent parolees, as well as others to provide information relevant to the application of a calmativ e in an agitated population, riot and/or hostage situation requiring deployment of a non-lethal technique.

This report highlights the pharmacological effects of a calmativ e, including a discussion of pharmacokinetic and pharmacodynamic principles of drug action in the central nervous system.

The Researchers identified the characteristics of an "ideal" calmativ e as a non-lethal technique to include:

- easy administration
- adaptable for administration via topical, subcutaneous, intramuscular, or oral routes
- rapid in onset
- most likely of short or limited duration
- production of approximately the same magnitude of calm (ranging from a less agitated, groggy, sleepy-like state to a stunned state of consciousness) in all individuals of similar body mass index and age range
- the effects should be reversible by a profile of rapid turnover and/or the availability of a selective antagonist to serve as an antidote
- the compound should be safely administered by an individual and free of prolonged toxicity to the individual(s) receiving the agent
- only be administered on a temporary basis
- produce side effects, if any, of short duration.

The Researchers noted that in identifying an optimum calmativ e for use as a non-lethal technique, the choice of agent for application in a field setting would depend upon the situation of the crisis requiring intervention. In this regard, wide ranges of potential agents were considered and it was noted that a series of calmativ es with different mechanisms of action, duration of effects and depths of "calm" might be appropriate for development. It was noted that drugs can be tailored to be highly selective and specific for known receptor (protein) targets in the nervous system with unique profiles of biological effects on consciousness, motor activity and psychiatric impact.

An extensive review of the medical research literature and several commercial sources of current pharmaceutical information were searched on topics carefully selected for their relevance to calmativ es. The CALMATIV E database generated includes over 7,000 references obtained in conducting this research. These results demonstrated that a large body of highly relevant information is available on calmativ e agents.

Several classes of compounds were identified from the CALMATIVE database as having high potential for use as a non-lethal calmative agent and include:

- benzodiazepines
- alpha₂-adrenoreceptor agonists
- dopamine D3 receptor agonists
- serotonin selective reuptake inhibitors
- serotonin 5-HT_{1A} receptor agonists
- opioid receptors and mu agonists
- neurolept anesthetics
- corticotrophin-releasing factor receptor antagonists
- cholecystokinin B receptor antagonists.

The discussion for each category of agent includes identification of specific compounds (typically receptor agonists and antagonists) as well as review of the clinical effects and the mechanism of action. In addition, each class of compounds and specific drugs were discussed in light of their proposed contribution as a non-lethal technique. The Researchers identified several drug classes (e.g. benzodiazepines, alpha 2-adrenoreceptor agonists) and individual drugs (diazepam, dexmedetomidine) found appropriate for immediate consideration as a non-lethal technique. Equally important, the Researchers identified many promising new developments that deserve further consideration with high potential as prototypical calmatives with availability in the near future.

It should be noted that the Researchers did not consider that a particular drug does not currently have a method of administration appropriate for immediate use as a non-lethal technique sufficient to disqualify a compound from further consideration as a non-lethal calmative agent, as the area of drug delivery continues to be a rapidly developing field. The Researchers has directed attention to several promising breakthroughs in improving drug delivery of macromolecular compounds and recommends this issue for further discussion.

The Researchers identified several drug classes for immediate consideration as a non-lethal technique.

Introduction

The Research Objective

The research objective for this project includes the accomplishment of the following:

- Define the advantages and limitations of pharmaceutical compounds as calmatives with potential use in non-lethal techniques.
- Provide a comprehensive survey of the medical literature utilizing pharmaceutical agents to produce a calm state with potential for use as a non-lethal technique. This information will provide a current database of the relevant literature on calmatives.
- Provide an in-depth review of selected calmatives identified by the literature search with high potential for further consideration as a non-lethal technique.
- Identify and recommend promising new areas in pharmaceutical drug development that are poised to uniquely meet the requirements of calmatives as non-lethal techniques.

... “calmatives” have undergone a remarkably rapid phase of growth.

The Subject of Review

Calmatives have potential for use in non-lethal techniques. Currently, the majority of non-lethal techniques involve the use of physical restraint, induction of acute physical pain, or other immobilization strategy. Chemical irritants, which include pepper spray or tear gas, serve to illustrate another series of approaches currently used in situations of crowd control. However, to date, the vast array of pharmaceutical compounds that produce a calm, non-agitated behavioral state, may have potential utility as non-lethal techniques, either alone or in combination with established approaches described above.

Since the mid 1960s, the availability of these pharmaceutical agents, herein termed “calmatives,” have undergone a remarkably rapid phase of growth. Indeed, the premier status of the US pharmaceutical industry in the world markets, combined with the exponential developments in the fields of pharmacology, neuroscience, anesthesia, and biotechnology fields, among others, has brought forth a diverse array of compounds that produce sedation and/or a calm state as either a primary or secondary effect. The challenge of assessing the potential use of calmatives as non-lethal techniques requires an initial screening of a broad array of potential candidates, followed by an in-depth assessment of compounds with features uniquely suited towards use as a non-lethal technique. The approach taken for this report on calmatives as non-lethal techniques was to a) define the characteristics of an “ideal” non-lethal calmative, b) provide a broad-based yet targeted review of medical based literature and commercial databases containing calmative properties with pharmaceutical agents to create a database resource, and c) conduct in-depth literature reviews of drug classes and prototype compounds

which may best meet the profile of a non-lethal calmative technique. In addition, this report highlights several new areas in pharmaceutical drug development with high potential for impact on development of non-lethal techniques.

Contribution of the Report on Calmatives

There is a need for non-lethal techniques with a high degree of specificity, selectivity, safety, and reversibility to avoid producing a lasting impairment to the subject(s) or individual(s) activating the technique. Consideration of the use of calmatives as non-lethal techniques is both timely and warranted.

There are numerous pharmaceutical agents with a profile of producing a calm-like behavioral state currently available in clinical practice. Moreover, wide arrays of new compounds with unique cellular and molecular mechanisms are under development by the pharmaceutical industry for their ability to produce calm- and tranquil-like states of behavior. Therefore, this report serves as an essential first step in identification of calmative pharmaceutical agents with potential utility as non-lethal techniques. The extensive survey of the literature conducted on calmatives serves to emphasize that the “time is right” with respect to considering pharmaceutical agents as appropriate new approaches to be incorporated in the design, enhancement, and implementation of non-lethal techniques.

In considering the application of calmatives as non-lethal techniques, The Researchers would also like to note that the use of these agents should be considered in an ethical context. While a review of the ethical principles and practices for use of non-lethal techniques is beyond the scope of this report, it is important to note that there are both national and international standards of conduct applicable to the practice of medicine and military conduct that may require consideration in the implementation of a calmative as a non-lethal technique.

Important Observations from Literature Search on Calmatives

Several key observations emerged during the course of conducting extensive literature research on calmatives. *First*, there is an explosion of new knowledge and developments in pharmaceuticals producing sedation and/or calm behavior as a direct and/or side effect. This wealth of information includes rapidly emerging developments in the fields of cellular and molecular biology, neuroscience, psychiatry and anesthesia, among others. *Second*, the goals of new drug development efforts, namely continued improvement in specificity, selectivity, safety and reversibility are the goals for improvements in non-lethal techniques. The compounds discussed that are under development by the pharmaceutical industry were selected for these advantages. Thus, new compounds are in “the drug development pipeline” that will have an improved ease of delivery, specific control of duration of effect, specific sites of action and other properties that may prove advantageous to design of innovative non-lethal techniques. *Third*, new classes of pharmaceutical agents and new compounds, are poised to meet the unique requirements of the non-lethal warfare arena. Ultimately, new compounds can be designed to better meet the requirements of non-lethal techniques for use in specific military and civilian situations.

There is a need for non-lethal techniques with a high degree of specificity, selectivity, safety, and reversibility...

...the goals of new drug development efforts, namely continued improvement in specificity, selectivity, safety and reversibility are the goals for improvements in non-lethal techniques.

Calmatives – Advantages and Limitations

Define the advantages and limitations of pharmaceutical compounds as calmatives with potential use as non-lethal techniques.

What Is A Calmative?

A wide variety of pharmacological approaches modulate mammalian behavior, including human, non-human primates and rodent species. Pharmacological compounds (or agents) producing a calm or tranquil behavioral state upon administration are termed “calmatives.” In most cases, the state of calm produced will, in part, depend on the existing behavioral state of the individual before the pharmaceutical agent is administered (e.g. agitated, aggressively violent) and the dose and route of drug to be administered, and the pharmacokinetic and pharmacodynamic properties of a given compound.

Pharmaceutical agents to be considered under the topic of “calmatives” will include compounds known to depress or inhibit the function of the central nervous system termed (depressants). There are several major classes of pharmaceutical compounds that fall under the category of depressants including sedative-hypnotic agents, anesthetic agents, skeletal muscle relaxants, opioid analgesics, anti-anxiety or anxiolytics, antipsychotics, antidepressants, and selected drugs of abuse. These pharmaceutical agents or “drugs” produce their effects by actions targeted to specific targets, typically receptor proteins that are located in the central nervous system, including the brain. While each of these drug classes have diverse mechanisms of effects on their target tissue (the nervous system), the range of drug-induced effects are dose-dependent. Depressant drugs can produce effects that range from anxiolytic, mild sedation, hypnotic and even coma and death as dependent upon the dose of drug administered and its spectrum of pharmacological effects.

Pharmaceutical compounds recommended for use as calmatives with high potential as non-lethal techniques will typically not be administered to produce deep sedation or hypnosis. Those recommended will relieve anxiety and mild sedation. Compounds selected for in-depth discussion will also have features that offer specific advantages in a non-lethal warfare setting (see following section on “An Ideal Non-Lethal Calmative”).

Pharmacological Effects of a Calmative

The pharmacological effects of a given compound are critically affected by its pharmacokinetic profile, which include its route of administration, rate of drug absorption and distribution, biotransformation, and excretion, as well as its pharmacodynamic profile, which determines its mechanism of action. For example,

*... “calmatives” ... include
... sedative-hypnotic agents,
anesthetic agents, skeletal
muscle relaxants, opioid
analgesics, anti-anxiety
antipsychotics,
antidepressants ...*

in order to understand the pharmacokinetic effects of a calmative agent, information is needed on how the drug has access to the brain (e.g. is it absorbed through the skin topically), information on variables which may enhance or inhibit the compound's distribution and/or metabolism (e.g. was the subject ingesting large quantities of alcohol), as well as the ability of the drug to be removed or cleared from the subject by excretion needs to be established. A critical factor with all drugs, including agents which act on the central nervous system, is their ability to enter the brain. The entry of drugs into cerebrospinal fluids and extracellular space of the central nervous system is restricted by the arrangement of endothelial cells described as "the blood brain barrier." Not only is the rate of cerebral blood flow an important factor in allowing a compound to reach the brain tissue, but the rate of diffusion of a drug into the central nervous system is affected by the size and the chemical charge or polarity of the agent.

In order to establish the pharmacodynamic profile of a compound, the chemical and physical interaction of a given drug with its target tissue must be delineated to understand the mechanism of action at a specific anatomical site in the central nervous system (CNS). Drug action in the CNS includes a critical role for receptors, which are proteins that serve as binding sites for endogenous regulatory ligands, including hormones and neurotransmitters. Receptors, and their associated effector and signal transduction systems, act as the integrators of extracellular information. Stimulation of a drug at its receptor by an agonist (termed receptor agonist) generally transmits a signal to an individual cell that, in turn, begins a cascade of cellular and molecular effects that alter the regulation of that cell. In turn, the specific effect of a drug on a given receptor may be chemically blocked by a targeted compound (termed receptor antagonist). As will be evident in the discussion of agents identified for use as potential calmatives in a non-lethal warfare setting, the specificity of the agent will be closely linked to actions as a receptor agonist or antagonist. The ability to stop or terminate the effective action of a given calmative may be linked to the administration of a specific receptor antagonist, which will, in turn, block the actions of the pharmaceutical agent at the target region in the brain or other central nervous system site.

Preclinical and Clinical Information on Calmatives

In order to obtain as complete information as possible on a given calmative agent with potential as a non-lethal technique, close attention to two distinctly different types of medical research literature was deemed essential — both preclinical and clinical research literature. Each type of research data provides a valuable and unique contribution to our current knowledge of calmative agents. Both preclinical and clinical research provides important information vital toward identification of calmatives that may be best suited for use as non-lethal techniques.

In the preclinical arena, research investigations are typically conducted in a variety of models ranging from *in vitro* isolated cell cultures to recording in brain slices or in anesthetized small animals. Often data obtained in a preclinical research setting is obtained using bacteria or yeast cell models. In addition, receptor pharmacology is often established in isolated membrane preparations. Each of these types of

preparations allow for the precise quantification of dose-response effects for a drug and provide the opportunity to explore cellular and molecular mechanisms of drug action; such experiments are simply not feasible or ethical in the intact human brain.

Results obtained from research conducted in a clinical setting provide additional information that is vitally needed in assessment of calmatative pharmaceutical agents. First, such data is required to demonstrate that a given drug will provide the desired behavioral effects. Moreover, while effective dose ranges can often be estimated in a preclinical research setting, the doses and routes of drug administration must be confirmed in a human population. Likewise, potential toxicities and side effects that may cause harm to an individual can be identified in a clinical research setting.

An additional feature of clinical literature, with particular relevance to the identification of pharmaceutical agents that may be effective non-lethal calmatives, is the use of research results obtained from specific patient populations. For example, pharmacotherapeutic approaches for the treatment of alcoholism, where patients are often experiencing withdrawal syndromes that include anxiety, agitation, disorientation and hallucinations, have identified serotonergic drugs and benzodiazepines as useful candidates in reduction of these withdrawal symptoms (see Objective 3). The application of serotonin active drugs as psychotropic agents for the treatment of patients suffering from social phobia, an anxiety-related disorder characterized by excessive fear in performance or interactive situations involving intensive evaluation by others, identified the effectiveness of treatment with buspirone and serotonin selective reuptake inhibitors (SSRIs) in these patient populations. Other selected patient populations, including individuals with obsessive-compulsive disorders, attention deficit hyperactivity disorders, or behavioral disturbances with dementia including disruptive behaviors and aggression, provide information directly relevant to the application of a calmatative in an agitated population, riot or hostage situation requiring use of a non-lethal technique.

An Ideal Non-Lethal Calmatative

In seeking to identify pharmaceutical agents useful as calmatives in a non-lethal technique, several characteristics may contribute to the profile of an "ideal" agent. The calmatative should be easy to administer and adaptable for administration via topical, subcutaneous, intramuscular or oral route. The onset of action for this compound should be fast (seconds to minutes) and most likely of short or of a limited duration (minutes). A given dose of the agent should produce approximately the same magnitude of calm (ranging from a less agitated, groggy, sleepy-like state to a stunned state of consciousness, sleep state, deep sleep or light anesthesia) in individuals of similar body mass index and age range. The effects of producing a calm state with this agent should be reversible either by a profile of rapid metabolism and elimination and/or the administration of a selective antagonist specifically designed to block the effects of the administered calmatative. The compound should be able to be safely administered by the individual utilizing the non-lethal technique and free of any prolonged toxicity to the individual receiving the agent; the calmatative should only be administered on a temporary, typically one time basis, and therefore produce side effects (headache, nausea, vomiting), if any, of

... research conducted in a clinical setting demonstrates that a given drug will provide the desired behavioral effects.

... selected patient populations... provide information directly relevant to the application of a calmatative in an agitated population, riot or hostage situation....

*"ideal" agent...
easy to administer...
onset should be fast...
short duration...
produce approximately the same magnitude of calm...
should be reversible...
free of any prolonged toxicity...*

short duration. Note that a series of calmatives may be appropriate for consideration with different mechanisms of action, duration of effects and different depths of “calm.”

In identifying an optimum or ideal calmative for use as a non-lethal technique, it should be recognized that the choice of agent for application in a field setting would depend upon the situation of the crisis requiring intervention. For example, an individual running towards you with a gun may pose an immediate threat or perhaps be trying to protect you; in contrast with this immediate threat are a group of hungry refugees that are excited over the distribution of food and unwilling to wait patiently. In these two cases the degree of “calm” required is vastly different in magnitude and the target populations are also different. In many cases the choice of administration route, whether application to drinking water, topical administration to the skin, an aerosol spray inhalation route, or a drug-filled rubber bullet, among others, will depend on the environment. It is also important to note that a pharmaceutical agent or drug cannot discriminate a target; whoever comes in contact with the agent will experience the intended dose-dependent pharmacological and physiological effects. However, drugs can be tailored to be highly selective and specific for known receptors and their biological effects on consciousness, motor activity and psychiatric profile.

*calmatives... might
necessitate medical
attention...*

As will be discussed under Objective 4, new and improved methods for administration of pharmacological agents are under continuing development. It should be noted, that the fact that a particular drug does not currently have a method of administration appropriate for immediate use as a non-lethal technique should not disqualify this compound from present and/or future consideration as a non-lethal calmative agent. The developments in this arena of drug application/administration will continue to emerge in a rapid fashion.

Required Use of Medical Attention with States of Unconsciousness

It should be recognized that all drugs, including calmatives that may cause unconsciousness might necessitate medical attention for the following:

- Ensure that the subjects did not “go to sleep” in positions that obstruct their airway,
- Check for the occasional person who may stop breathing (many medical reasons in the unhealthy, the elderly and very young but very unlikely in fit, healthy soldiers or other individuals who may not be the target population),
- Avoid injuries due to the fall of “falling asleep” (bump to head on object or floor),
- Administer an antidote which may require medical attention; and
- Determine how long to monitor and provide medical attention as dependent upon the route of administration and dose of the active calmative and/or antidote/selective antagonist.

Specific Advantages of Calmatives Over Other Non-lethal Techniques

Are there advantages to the production of a calm state in a non-lethal technique versus the use of blunt trauma and/or compliance through pain? One area of

consideration is that blunt trauma has an incidence of organ damage, which may include the eyes, liver, kidney, spleen, heart and brain, that may be permanent or even death. Other methods of inflicting pain for control of an individual are sometimes socially unacceptable. In contrast, a pharmaceutical agent may be administered in a discrete manner to a selected individual or a drug agent may be selected with a known duration of effect. Moreover, much like compliance produced with blunt trauma or other restraint method, virtually all individuals will respond in a dose-dependent manner. The limitation to the use of calmatives in a non-lethal technique may, therefore, be relatively few.

*...virtually all individuals
will respond in a dose-
dependent manner.*

Literature Survey - Methodology

Provide a comprehensive survey of the medical literature identifying pharmaceutical agents that produce a calm state with potential for use as a non-lethal technique. This information will provide a database of current relevant calmative literature.

The Search Process

A comprehensive and intensive search of the published research literature was conducted on pharmaceutical agents with potential utility as calmatives. The overall strategy of the search process was to identify key topics (e.g. calmatives, sedation), drug classes (e.g. benzodiazepines, opioids), drugs (e.g. RB101, midazolam), and behaviors (e.g. aggression, agitation), and then systematically search a wide variety of biomedical research literature databases. On a weekly and sometimes daily basis, results of searches were reviewed by the Researchers and further refined and redirected. In addition to the Researchers, searches were conducted by two postdoctoral level individuals with backgrounds in neuropharmacology and with assistance from a reference librarian at Penn State University. As the volume of material is so extensive under many of the topics, the task required constant assessment of the quality of the reference material and relevance to the non-lethal technique requirements.

In addition, commercially available databases were searched, including DIALOG.com and IDDB.com (Investigations Drug Development Base). These sources were carefully selected for high quality and their access and emphasis on the newest developments in drug development, including compounds undergoing drug discovery and Phase I, II or III clinical investigation trials. Thus, the search process utilized the most up-to-date information currently available on pharmaceutical compounds under development. It should be noted that the Researchers did not have access to any proprietary information that had not yet been published or protected by patent-related activity; such data access would require individual disclosures and confidentiality agreements beyond the scope of the present investigation.

Records were maintained on all searches, including key words, dates of searches, databases searched, outcomes, and hardcopies.

Profile of the Calmative Database

All references identified in the search process are included in the database termed CALMATIVE; this database includes over 7,800 references.

References and abstracts were placed in a Reference Manager format that is designed for use with Windows 98/95/NT. The CALMATIVE database is included with this report on a formatted ZIP drive and is available in printed format upon request.

...the search process utilized the most up-to-date information currently available on pharmaceutical compounds under development.

In addition, to improve the ease of subsequent use of the CALMATIVE database, a second formatted database, termed CALM TOPICS database was developed. This database which includes identification of subtopics and themes identified for references included in the master CALMATIVE database. The topics and themes included in CALM TOPICS are listed in Table 1.

This selected database provides a comprehensive survey of the current medical literature utilizing pharmaceutical agents to produce a calm state with potential for use as a non-lethal technique (30 April 2000).

TABLE 1. TOPICS AND THEMES OF THE CALM TOPIC DATABASE.

1. Calmatives	2. Aggression
3. Antipsychotics and Aggression	4. Treatment of Agitation
5. Treatment of Panic	6. Animal Model of Panic
7. Lactate Induced Panic	8. Alnespirone
9. Lesopitron	10. Benzodiazepine
11. Benzodiazepine – Mechanism	12. Anxiety in Mice
13. Anxiety in Rats	14. Anxiety in Mammals
15. Anxiety in Humans	16. Animal Screening
17. Disinhibit	18. Zopiclone
19. Zolpidem	20. Zaleplon
21. Flumazenil	22. Resiniferatoxin
23. Divalproex	24. GABA _A Receptor & Deficient
25. GABA _A Receptor & Mutant	26. GABA _A Receptor & Tansgenic
27. GABA _A Receptor & Anxiety	28. GABA _A Receptor & Sedation
29. Alprazolam	30. Nefazodone (Panic & Nefax – Venlafax – Fluvox)
31. Valproate & Panic Disorder Aggression	32. Venlafaxine, Fluvoxamine, Nefazodone, Mirtazapine & Aggression
33. PTSD – Drug Therapy	34. Lorazepam
35. Midazolam (Versed)	36. Flunitrazepam
37. Etizolam	38. Short-acting Benzodiazepines
39. Alpha2 Agonist & Agitation	40. Alpha2 Agonist & Panic
41. Alpha2 Agonist & Postoperative	42. Alpha2 Agonist & Sedation
43. Clonidine & Sedation	44. Clonidine & Agitation
45. Clonidine & Pain	46. Clonidine & Postoperative
47. Dexmedetomidine	48. Dopamine Receptor Agonist & Schizophrenia
49. Dopamine Receptor (DAR) Agonist & Aggression	50. Dopamine Receptor (DAR) Antagonist & Aggression
51. Dopamine Receptor (DAR) Antagonist & Agitation	52. Dopamine Receptor (DAR) Antagonist & Schizophrenia
53. D3 & PCP	54. PCP Psychosis
55. PD 128907 (D3 Agonist)	56. D3 Receptor & Schizophrenia
57. D3 Receptor & Psychosis	58. D4 Receptor & PCP Psychosis
59. Phencyclidine & Dopamine	60. Phencyclidine & Mechanism
61. Oleamide	62. 5-Hydroxytryptamine Moduline
63. Sertraline Aggression	64. Buspirone & Anxiety
65. Buspirone & Aggression/Mood/Combat	66. Buspirone & Tranquilizers
67. Buspirone & Motor Activity	68. Buspirone & Impulse Control Disorder
69. Buspirone & Phobic Disorders	70. Buspirone & Alcohol Drinking
71. Buspirone & 5HT Antagonists	72. Buspirone & DA/D ₂ receptors
73. Carfentanil	74. Propofol (Diprivan)
75. Opioid & Impulse Control	76. Opioid & Aggression
77. Mu Agonists	78. Orphanin Fq
79. Sigma & Aggression	80. Anandamide
81. CRF Antagonists	82. CCK
83. CCK Receptors	84. CI-1015 (CCKB Antagonist)
85. CI-988	86. CR-2945 I (CCKB Antagonist)

87. Human, Hypercortisolism & Aggression	88. Human Hypercortisolism
89. Salway Cortisol	90. Neuropeptides & Anxiety
91. Prohormone Convertase/Proprotein Convertase	92. Neuropeptide Y Antagonist
93. Neuropeptide & Antagonist – BIBP3226	94. Neuropeptide Y & Agonist
95. Neuropeptides/Substance P Antagonists	96. Urocortin & Antagonist
97. Oxytocin & Agonist	98. GHB
99. Neuroleptic	100. Neuroleptanalgesia
101. Risperidone	102. Tramadol
103. Citocoline	104. MRI & Rodent
105. Nitric Oxide Synthase – Brain	106. RO 48-6791
107. Sevoflurane	108. Desflurane
109. Isoflurane	110. Butanediol
111. Gastrin Receptor	112. Author – Reinscheid, R.K.
113. Author – Koster, A.	

Selected Calmatives – Results

Provide an in-depth review of selected calmatives identified by the literature search with high potential for further consideration as a non-lethal technique.

Rationale for Topics Selected

The pharmaceutical drug classes and agents selected that are highlighted in the following section have been chosen for an appropriate action as a calmative (Table 2). These compounds have been demonstrated, indicated and/or are being developed to treat agitated, aggressive and/or anxiety-related behaviors and, hence, result in a calm state. A range of drugs with distinctly different cellular and molecular mechanisms of action with diverse biological and physiological effects has also been included. We recognize that “an ideal calmative” may well be dependent upon the situation in which it is ultimately deployed. Therefore, it is important that all promising candidate calmatives and options be explored.

For each drug class and pharmaceutical agent chosen for further discussion, a brief summary of characteristics is provided, followed by a more in-depth discussion with selected references.

TABLE 2. SELECTED CALMATIVES.

Drug Class	Selected Compounds	Site of Action
Benzodiazepines	diazepine (Valium)	GABA receptors
	midazolam (Versed)	
	etizolam	
	flumazenil (antagonist)	
Alpha ₂ Adrenergic Receptor Agonists	clonidine	Alpha ₂ -adrenergic receptors
	dexmedetomidine (Precedex)	
	fluparoxan (antagonist)	
Dopamine D3 Receptor Agonists	pramipexole	D3 receptors
	CI-1007	
	PD 128907	
Selective Serotonin Reuptake	fluoxetine (Prozac)	5-HT transporter
	sertraline (Zoloft)	
	paroxetine	
	WO-09500194	
Serotonin 5-HT _{1A} Receptor Agonists	bupirone (Buspar)	5-HT _{1A} receptor
	lesopitron	
	alnespirone	
	MCK-242	
	WAY-100,635	
	oleamide	
Opioid Receptors and Mu Agonists	morphine	Mu reception; enzymatic
	carfentanil	
	naloxone (antagonist)	
Neurolept Anesthetics	propofol (di-iso-propylphenol)	GABA receptor

Drug Class	Selected Compounds	Site of Action
	droperidol and fentanyl combination (Innovar)	DA, NE, and GABA receptors
	phencyclidines (Ketamine)	Opioid receptors
Corticotropin-Releasing Factor Receptor Antagonists	CP 154,526 (antagonist) NBI 27914 (antagonist) CRF-BP (binding protein)	CRF receptor
Cholecystokinin B Receptor Antagonists	CCK-4 CI-988 (antagonist) CI-1015 (antagonist)	CCKB receptor

Benzodiazepines

SELECTED COMPOUNDS:

- diazepam (Valium)
- midazolam (Versed)
- etizolam
- flumazenil (antagonist)

CLINICAL EFFECTS:

Benzodiazepines are used as calming agents, for treatment of anxiety (anxiolytics), amnesia, pre-operative sedation and induction of general anesthesia. A side effect is that these agents depress respiration and the cardiovascular system. Benzodiazepines are used extensively on a daily basis, as oral and intravenous agents, and fall into 3 categories:

1. Ultra-short acting agents (midazolam) with therapeutic half-lives measured in minutes are used for sedation and anesthetic induction.
2. Intermediate acting (lorazepam) with half-lives of 10-18 hours, are used for short-term relief of anxiety symptoms.
3. Long acting agents (diazepam) with elimination half-lives of 100 hours or more are used for treatment of anxiety disorders and adjuncts in managing seizure disorders.

Paradoxical reactions, such as anger, hostility, and mania have been reported with presently available benzodiazepines.

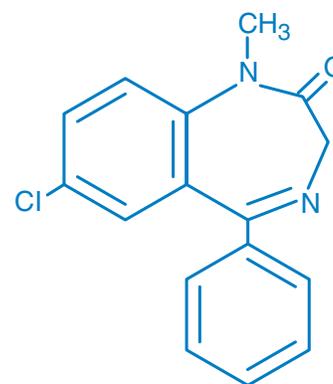
Flumazenil, is available as an antidote to benzodiazepines and has been used as an effective competitive antagonist in cases of toxicity or overdose.

MECHANISM OF ACTION:

Benzodiazepines stimulate and enhance gamma-aminobutyric acid (GABA) which is the major inhibitory neurotransmitter in the central nervous system (brain and spinal cord).

There are several GABA receptors (GABA_A, GABA_B, GABA_C) which have further sub-units and isotypes of sub-units (see full description). Different regions of the brain have receptors of differing combinations of subtypes, thus conferring different pharmacological properties in various areas of the central nervous system.

*Benzodiazepines...
depress respiration and the
cardio-vascular system.*



Diazepam (Valium)

Due to the existence of differing GABA receptor sub-units in various brain regions, it may be possible to design benzodiazepines that mediate sedative effects without producing respiratory and cardiovascular depression and also lacking the paradoxical effects of stimulation.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:
Benzodiazepines are prototypical calmatative agents with varying profiles from rapid onset and short-acting, through intermediate acting, to very long-term effects. The agents can be administered by a variety of routes, including oral and parenteral (intramuscular and intravenous). The benzodiazepines have synergistic effects with several other pharmaceutical agents with direct and indirect GABA effects (e.g. barbituates and narcotics), leading to lower doses, and enhanced safety, for both groups of agents.

This literature search has indicated that benzodiazepines (and all GABA receptor agonists) have a major potential use as non-lethal technique calmatives.

DISCUSSION:

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. Several receptors, termed GABA_A, GABA_A, and GABA_C have been identified as endogenous targets for GABA binding. Both GABA_A and GABA_C receptors exist as receptor-chloride ion channel hetero-oligomeric macromolecular complexes, while GABA_B receptors are members of the G-protein coupled receptor super-family.

Many pharmacologic agents with anxiolytic and sedative properties are known to interact with GABA_A receptors. This receptor is comprised of at least three different subunits, termed alpha, beta and gamma. Several different isotypes for each of the subunits have been identified, including six different alpha subunits, 4 beta subunits, and three gamma subunits. The stoichiometric arrangement of these subunits is presently unknown. However, receptors in different regions of the brain are comprised of differing combinations of subtypes, thus conferring different pharmacologic properties to the receptor-ion channels in various areas of the central nervous system.

Experimental analysis has shown that GABA binds to sites on either the alpha or beta subunits of the GABA_A receptor complex. When GABA is bound to its receptor, chloride conductance through the ion channel portion of the receptor is initiated. As chloride ions pass through the ion channel, neuronal membrane hyperpolarization results, making it more difficult for neurons to reach their threshold potential for neuronal firing. Ultimately, GABA binding to GABA_A receptors results in a decreased firing rate of neurons in the central nervous system.

Sedative-hypnotic agents, such as benzodiazepines act at GABA_A receptors. Benzodiazepines do not substitute for GABA, however, they augment the effects of GABA by binding to the gamma subunit of the receptor-ion complex. Benzodiazepines enhance chloride conductance initiated by GABA's interaction

with GABA_A receptors by increasing the *frequency* of ion channel opening events. (It is worth noting that older generation sedative-hypnotics such as drugs in the barbiturate class also act at GABA_A receptors by increasing the *duration* of GABA-gated ion channel openings. Due to their low margin of safety, barbiturates have been largely replaced by the safer benzodiazepine class of agents.)

Clinically, benzodiazepines have been used as calming agents for a wide variety of therapeutic purposes including sleep induction, adjuncts to surgical anesthesia, epilepsy, depression of polysynaptic musculoskeletal reflexes, and treatment of anxiety states. A drawback to usage of benzodiazepines is that even at therapeutic doses, respiratory and cardiovascular depression may occur, probably as a result of action on medullary respiratory and vasomotor centers.

Although benzodiazepines share a common chemical structure, that of a 1,4-benzodiazepine, the side-groups added to each agent confer slightly different properties to the various compounds. These substitutions alter both the pharmacologic properties of individual drugs, rendering some agents more efficacious in achieving various calming states than others, as well as the pharmacokinetic properties of the drugs. Pharmacokinetically, benzodiazepines vary in their rates of onset of activity and their duration of action, which is influenced by route of elimination and the presence or absence of active metabolites. Chlorazepate and diazepam have a very fast onset of action that terminates rather slowly resulting in elimination half-lives of 100 hours or more. The properties of these agents render them well suited for managing anxiety disorders, particularly related to acute alcohol withdrawal, and as adjuncts in management of seizure activity. Other agents such as alprazolam and lorazepam are relatively short acting. The onset of activity of these agents has been termed “intermediate” and these compounds have therapeutic half-lives of approximately 10-18 hours. These agents are indicated for the short-term relief of anxiety symptoms. Still other benzodiazepines are ultra-short acting. These drugs have a very rapid onset of action, which terminates in a matter of minutes. Clinically, midazolam is an example of an ultra-short acting benzodiazepine. Midazolam is useful for sedation and anesthetic induction, processes which may occur in as little as two to five minutes following intravenous injection.

Alprazolam is administered orally and used clinically to manage anxiety or panic disorders. Although, primarily used as a calming agent, paradoxical reactions such as severe anger, hostility, and mania have been reported with this agent. While benzodiazepines are considered to have a wider margin of safety than barbiturates, death is conceivable with high enough doses due to depressive effects of these agents on medullary functions. However, there are no well-documented fatal overdoses resulting from oral use of benzodiazepines alone; most fatalities indicate benzodiazepines as only a component to overdose in combination with multiple drug injections.

In contrast to alprazolam, which is administered only by the oral route of administration, midazolam is typically given as an injection. Midazolam has found

... benzodiazepines have been used as calming agents for a wide variety of therapeutic purposes including sleep induction, adjuncts to surgical anesthesia, epilepsy, depression of polysynaptic musculoskeletal reflexes, and treatment of anxiety states.

use primarily as a preoperative sedative agent although it is also administered for sedation, anxiolysis and amnesia prior to or during short diagnostic procedures. Midazolam may also be used as an induction agent for general anesthesia. An off-label use of midazolam involves treatment of epileptic seizures. Midazolam has been associated with respiratory depression and respiratory arrest leading to death or hypoxic encephalopathy. Although benzodiazepines have the potential to produce respiratory depression and cardiovascular collapse in overdose situations, their safety profiles are considerably safer than older generation sedative agents. Furthermore, in cases of toxicity or overdose, a benzodiazepine receptor antagonist, flumazenil, is available that can be utilized to reverse adverse effects of benzodiazepine over-administration.

Newer, investigational short-acting benzodiazepines are currently under investigation for potential roles as calming agents in various types of anxiety, depressive disorders, and even schizophrenia. Etizolam, a short-acting benzodiazepine with elimination kinetics between those of short-intermediate and ultra-rapidly eliminated benzodiazepines (Fracasso et al., 1991), is one such agent. Following administration of etizolam to patients suffering from panic disorders, significant improvements were seen in the following areas: chronic anxiety, phobic ideas, depressive symptoms and episodic anxiety (Savoldi et al., 1990). Additionally, etizolam has also been used successfully to suppress schizophrenic auditory hallucinations refractory to antipsychotic treatment (Benazzi et al., 1993). Another investigational benzodiazepine is Ro 48-6791. This agent has been found to be comparable with midazolam, with a slightly shorter duration of action (Tang et al., 1996; Dingemans et al. 1997). Ro 48-6791 is currently in phase II clinical trials for evaluation as an anesthesia induction agent. Further trials are needed to more fully evaluate the effectiveness of these compounds in mediating various calmative states. Their clinical utility, in comparison with other benzodiazepines, remains to be determined.

In addition to the search for new short-acting compounds, another area of benzodiazepine research is focused on the distinct pharmacologic properties mediated by various combinations of GABA_A receptor subunits. In experimental systems, the intrinsic activity of benzodiazepine receptor ligands has been found to vary with different combinations of receptor subtypes (Knoflach et al., 1993). The degree of receptor modulation appears to be influenced primarily by the type of alpha subunit present. Several agents including bretazenil and imidazenil are presently being evaluated for effectiveness as calming agents in various disorders by taking advantage of their selectivity for various GABA_A receptor subunit combinations.

With GABA_A receptor subunits expressed differentially in various brain regions, it may be possible to design benzodiazepines that mediate sedative or anxiolytic effects without causing respiratory and cardiovascular depression. Much information remains to be garnered regarding the properties of various combinations of GABA_A receptor subtypes in the brain, both with respect to their localization patterns within the central nervous system and their pharmacological properties.

Further experimental research in this area may aid in the design of drugs that mediate calming properties without eliciting other adverse events.

SELECTED REFERENCES:

- Benazzi F, Mazzoli M and Ressi E (1993) Auditory hallucinations suppressed by etizolam in a patient with schizophrenia. *Canadian Journal of Psychiatry* **38**:574-575.
- Dingemans J, van Gerven JM, Schoemaker RC, Roncari G, Obery JJ, van Oostenbruggen MF, Massarella J, Segala P, Zell M and Cohen AF (1997) Integrated pharmacokinetics and pharmacodynamics of Ro 48-6791, a new benzodiazepine, in comparison with midazolam during first administration to healthy male subjects. *British Journal of Clinical Pharmacology* **44**:477-486.
- Francasso C, Confalonieri S, Garattini S and Caccia S (1991) Single and multiple dose pharmacokinetics of etizolam in healthy subjects. *European Journal of Clinical Pharmacology* **40**:181-185.
- Knoflach F, Drescher U, Scheurer L, Malherbe P, Mohler H (1993) Full and partial agonism displayed by benzodiazepine receptor ligands at recombinant gamma-amino butyric acid A receptor subtypes. *Journal of Pharmacology and Experimental Therapeutics* **266**:385-391.
- Savoldi F, Somerzini G and Ecari U (1990) Etizolam versus placebo in the treatment of panic disorder with agoraphobia: A double-blind study. *Current Medical Research Opinion* **12**:185-190.
- Tang J, Wang B, White PF, Gold M and Gold J (1996) Comparison of the sedation and recovery profiles of Ro 48-6791, a new benzodiazepine, and midazolam in combination with meperidine for outpatient endoscopic procedures. *Anesthetic Analgesia* **89**:893-898.

Dexmedetomidine... produces good sedation, some psychomotor impairment and moderate analgesia without cardiovascular compromise.

Alpha₂ Adrenergic Receptor Agonists

Selected Compounds:

- clonidine
- dexmedetomidine (Precedex)
- fluparoxan (antagonist)

CLINICAL EFFECTS:

Alpha₂ adrenoceptor agonists cause sedation, anxiolysis and enhance the effects (synergism) of both general anesthetic and local anesthetic agents. Dexmedetomidine, in low doses, produces good sedation, some psychomotor impairment and moderate analgesia without cardiovascular compromise.

MECHANISM OF ACTION:

The agents are selective agonists at the alpha₂ adrenoceptor located in the brain and spinal cord. Clonidine (as the original drug), is five-fold less selective than dexmedetomidine, causing transient hypotension due to stimulation of the alpha₁ adrenoceptor in the peripheral vascular system. Therefore, clonidine should not receive further consideration.

Dexmedetomidine acts selectively on the alpha_{2A} adrenoceptor by opening inwardly-rectifying K⁺ channels distributed in locus coeruleus of the central nervous

system and causes an anti-nociceptive (anti-pain) effect. Dexmedetomidine was originally developed as a sedative-analgesic for veterinary medicine. It was released in March 2000 in the USA as an “anesthetic” for sedation in intensive care patients as an anxiolytic agent.

Dexmedetomidine potentiates several anesthetic agents (e.g., thiopentone induction requirement is reduced by 23%, and isoflurane requirements are reduced by more than 90%), as well as decreases the requirements for opioids (e.g., pentazocine requirement for post operative pain relief is decreased by 70%). Furthermore, dexmedetomidine significantly potentiates electro-acupuncture to suppress cerebral evoked potential and, therefore, has potential for use with “sticky shocker” (see below). This α_2 adrenoreceptor agonist also attenuates the side effects of ketamine, including cardio-stimulatory as well as delirium effects and this interaction may be important for non-lethal technique use.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:
Dexmedetomidine can be administered as an intravenous, intramuscular and transdermal agent. Used in conjunction with most other sedative agents, this drug markedly (23-90%) reduces the dose requirement for the primary agent, often reducing side effects leading to increased safety of the mixture of pharmaceutical agents.

Used in conjunction with most other sedative agents, this drug markedly (23-90%) reduces the dose requirement for the primary agent, often reducing side effects leading to increased safety of the mixture of pharmaceutical agents.

The interesting phenomenon of potentiating electro-acupuncture opens the possibility that use of this agent in conjunction with existing (e.g., sticky shocker) and proposed non-lethal techniques (electro-magnetic waves) is warranted. The concept could be considered that exposure of a group first to the pharmacological agent (leading to mild sedation and sleepiness), can then be added to another directed non-lethal technique. This approach would address effects on the few individuals where an average dose of the pharmacological agent did not have the desired effect. This approach offers the advantage of avoiding the use of a higher dose of pharmacological agent, which may prove to be too much for some individuals.

Fluparoxan is under development as an antidote (competitive antagonist) for α_2 adrenoreceptor agonists. The availability of a highly selective antagonist will permit the rapid reversibility of drug-induced effects and enhance the safety profile of α_2 adrenoreceptor agonists as non-lethal calmative techniques.

Dopamine D3 Receptor Agonists

SELECTED COMPOUNDS:

- pramipexole
- CI-1007
- PD 128907

CLINICAL EFFECTS:

The D3 receptor agonists have been extensively investigated as calming agents – this is their presumed mechanism of action as anti-psychotic agents. They have yielded promising results in calming abnormal states produced by agents such as

phencyclidine (PCP). D3 receptor agonists have also been found to be useful in blocking convulsant effects of agents such as cocaine. Multiple other uses for D3 and other dopamine receptor agonists have also been investigated (see expanded section).

MECHANISM OF ACTION:

Dopamine is the predominant catecholamine neurotransmitter, or chemical, in the brain that transmits messages between nerve cells. In the central nervous system, dopamine has been implicated in the control of a variety of functions including regulation of motivation and positive reinforcement, locomotor activity, cognition, emotion, as well as endocrine regulation.

Four main dopaminergic pathways exist in the brain with associated behavioral functions:

- coordination of voluntary movements (nigrostriatal pathway)
- motivational, emotional and cognitive functions (mesolimbic and mesocortical pathways)
- endocrine functions (tuberoinfundibular pathway)

Dopamine exerts its actions in the central nervous system by interacting with five different receptors termed D1 through D5.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

The dopamine D3 receptor agonists are of great interest as a non-lethal technique due to the calmatative effects of these agents, as well as effects on motivation and on locomotion. Furthermore, the ability of the D3 receptor agonists to calm a psychotic subject, especially those with emotional behavior due to drugs such as PCP (phencyclidine) and cocaine, add further value to these D3 receptor agonists.

DISCUSSION:

Dopamine is the predominant catecholamine neurotransmitter, or chemical, in the mammalian brain that transmits messages between neurons. In the central nervous system, dopamine has been implicated in the control of a variety of functions including regulation of locomotor activity, cognition, emotion, positive reinforcement, and endocrine regulation.

Four main dopaminergic pathways exist in the mammalian brain to account for dopamine's central activity: the nigrostriatal pathway, the mesolimbic pathway, the mesocortical pathway, and the tuberoinfundibular pathway. The nigrostriatal pathway originates with dopamine-producing cells in the substantia nigra pars compacta which project to the dorsal striatum, a structure associated with the coordination of voluntary movements. Degeneration of dopaminergic neurons within this pathway have been implicated in Parkinson's disease. The mesolimbic pathway originates in the ventral tegmental area and projects to the nucleus accumbens, olfactory tubercle, and parts of the limbic system (septum, amygdaloid complex, and piriform cortex). Like the mesolimbic pathway, the mesocortical dopaminergic pathway also has its origins in the ventral tegmental area but projects to the frontal,

D3 agonists... calm a psychotic subject, especially those with emotional behavior due to drugs such as PCP (phencyclidine) and cocaine, add further value to these D3 receptor agonists.

cingulate, and entorhinal cortices. Together, the mesolimbic and mesocortical pathways are involved in emotional, motivational and cognitive functions. It is believed that aberrant dopaminergic neurotransmission within the mesocorticolimbic pathways underlies the disease of schizophrenia. The tuberoinfundibular pathway is comprised of dopamine-producing cells in the arcuate nucleus which release dopamine into the median eminence. Dopamine exerts an inhibitory effect on prolactin secretion; therefore, in the tuberoinfundibular pathway, dopamine regulates endocrine functions.

Dopamine exerts its actions in the central nervous system by interacting with five different receptors (D1, D2, D3, D4, and D5) which arise from five distinct genes. These receptors can be further separated into subfamilies based on pharmacology, sequence homology, and biochemistry. D1 and D5 receptors are similar to each other with regard to amino acid sequence, anatomical distribution, and biochemical pathways activated following receptor stimulation. As a result of their similarities, D1 and D5 receptors are commonly referred to as D1-like receptors.

Within the central nervous system, both D1 and D5 receptors are expressed at high levels in the cortex, striatum, and hippocampus. Ultrastructurally, D1 receptors are predominantly located on dendritic spines whereas, D5 receptors are preferentially expressed on dendritic shafts. This differential distribution suggests that although D1 and D5 receptors possess similar pharmacology they are not functionally redundant receptors. Stimulation of D1-like dopamine receptors in the central nervous system results primarily in activation of adenylate cyclase, although there is also evidence to suggest a role for D1-like receptors in the modulation of intracellular calcium levels as well.

D2, D3, and D4 receptors are also grouped together and are commonly referred to as D2-like dopamine receptors. D2-like dopamine receptors are structurally homologous to one another and biochemically inhibit adenylate cyclase activity. While second messenger activity varies from cell line to cell line, D2-like receptors are also capable of mediating changes in intracellular calcium levels, modulating potassium levels, potentiating arachidonic acid release, and activating a sodium/hydrogen ion exchanger. D2 receptors are predominantly expressed in the striatum, although their expression is widespread and is also located in the nucleus accumbens and various cortical and subcortical regions. In contrast to the widespread localization of D2 receptors, the localization of D3 and D4 receptors is restricted primarily to limbic regions of the brain. D3 receptors are predominantly located in the nucleus accumbens, olfactory tubercle, islands of Calleja and lobules 9 and 10 of the cerebellum. D4 receptors are highly expressed in the frontal cortex, amygdala, hippocampus, and hypothalamus. Additionally, splice variants of D2 and D3 dopamine receptors have also been identified. The D2 splice variant, termed D2S, appears to function in a manner similar to its full-length counterpart; however, the splice variants identified for D3 receptors appear to either be non-functional or to regulate cell-surface expression of D3 receptors in a dominant negative manner. Presently, the functional effects that co-expression of D3 receptors with D3-splice variants have on dopaminergic neurotransmission is under investigation.

Physiologically, D3 receptors appear to play an inhibitory effect on locomotion. D3-preferring agonists inhibit locomotor activity, while D3-preferring antagonists stimulate locomotor activity in experimental animals. Furthermore, mice genetically engineered to be D3 receptor-deficient are hyperactive compared to control mice. For the most part, however, the role of D3 receptor in the physiology of the dopaminergic system is largely unknown. Because D3 receptors are specifically expressed in limbic and cortical regions involved in the control of cognition and emotion, they are attractive targets for new generations of antipsychotic medications.

An association has recently been made with regard to schizophrenia and abnormally spliced D3 receptors. Post-mortem findings have indicated that in several brain regions, schizophrenic patients express primarily D3nf, a D3 receptor splice variant, and very little if any D3 receptors. This finding, coupled with the recent demonstration that when co-expressed *in vitro* D3nf may prevent D3 receptors from reaching the cell surface, suggests that abnormal function/regulation of D3 receptors may be involved with psychoses associated with schizophrenia.

Recently, a FDA-approved D3-preferring receptor agonist indicated for the treatment of Parkinson's disease, pramipexole, was used successfully in conjunction with traditional neuroleptic treatment in schizophrenic patients. Positive as well as negative symptoms of schizophrenia were reportedly diminished by 22-62% when pramipexole was added to haloperidol drug therapy (Kaspar et al., 1997). Another agent, CI-1007, a putative dopamine autoreceptor agonist and partial D2/D3 receptor agonist, is currently under development specifically for the treatment of schizophrenia (Sramek et al., 1998). Thus far, these studies have enrolled only a small number of patients and have only been conducted for short time periods. More extensive experimentation examining the effectiveness of D3 receptor agonists as calming agents will be required to elucidate the mechanism of their antipsychotic action.

The calming nature of D3 receptor agonists in treating diseases such as psychoses at first seems to be counter-intuitive given the current repertoire of drugs used as antipsychotic agents, which have historically been D2-like receptor *antagonists*. One school of thought on the paradoxical effectiveness of D3 agonists as calming agents is related to the localization of D3 receptors within neuronal synapses. Within the mesocorticolimbic system, D3 receptors are located pre-synaptically and function as autoreceptors. As such, one functional role of D3 autoreceptors is to regulate dopamine release in an inhibitory manner. It could be proposed that if D3 receptors are not properly localized within dopaminergic synapses, they might not mediate appropriate autoreceptor function. Failure of proper D3 receptor function could be due to faulty dopaminergic circuitry as a result of D3 receptor-second messenger uncoupling, inappropriate D3 receptor protein-protein interactions, or lack of D3 receptor cell surface expression due to over-expression of D3nf or other D3 receptor splice variants. Failure of D3 autoreceptors to relay appropriate negative feedback in response to dopamine may result in overactive dopamine release. Excessive dopamine can, in turn, be blocked by treatment

with dopamine receptor antagonists (current therapeutic approach to schizophrenia treatment). Alternatively, if this hypothesis is correct, pharmacologic *agonists* specifically targeting D3 autoreceptors might also prove effective as calmatives.

Another D3 receptor agonist, PD12890, has also yielded promising results in animal models of schizophrenia. Experimental models of schizophrenia in rats may be induced following administration of various pharmacologic agents including phencyclidine (PCP), apomorphine, or dizocilpine. Administration of PD128907 was found to produce an unusual sedative effect on stereotyped behavior in several rat models of schizophrenia (Witkin et al., 1998). This sedative effect was comparable in efficacy to clozapine and more efficacious than that produced by haloperidol, two commonly used anti-psychotic medications. Additionally, PD128907 caused no movement disorders, a common, severe side effect of haloperidol treatment (Witkin et al., 1998) nor was PD128907 associated with life-threatening blood disorders, a limiting factor of clozapine therapy.

An additional mechanism of action of the D3 receptor agonists pramipexole and PD 128907 may also play a role in their antipsychotic activity. These D3 receptor agonists are potent antioxidants and have been shown to be neuroprotective, preventing neuronal death caused by high local concentrations of dopamine. Furthermore, these D3 receptor agonists were shown to enhance the growth of dopaminergic neurons (Ling et al., 1999). The neuroprotective and antioxidant properties of these D3 receptor agonists aids in explaining the benefit that these agents provide in animal models of PCP-induced psychosis. The frequently abused drug PCP appears to induce psychosis by modulation of several neurotransmitter systems, acting both as a NMDA glutamate receptor antagonist and an inhibitor of dopamine reuptake. Together, this results in increased concentrations of dopamine in neuronal synapses following PCP administration, which is deleterious to the survival of neurons. Therefore, it appears that D3 receptor agonists may exert their calming, antipsychotic actions within the central nervous system via several different and unique mechanisms.

Another interesting feature of the D3 receptor agonist PD128907 is that this agent has also been found effective in blocking convulsant and lethal effects of cocaine administration (Witkin and Gasior, 1998). The mechanisms by which a D3 receptor agonist may exert such dramatic calming properties is currently unknown and thus more experimentation targeted at understanding the physiologic function of D3 receptors is warranted. Further development of D3 dopamine receptor specific agonists and antagonists will be required to elucidate the calming effects of D3 receptor agonists such as PD128907, CI-1007, and pramipexole. Additional clinical studies will also be required in order to fully ascertain the pharmacologic applications for which D3 receptor agonists will be of use as calmative agents.

SELECTED REFERENCES:

- Kasper S, Barnas C, Heiden A, Volz HP, Laakmann G, Zeit H and Pfolz H (1997)
Pramipexole as adjunct to haloperidol in schizophrenia: Safety and efficacy.
European Neuropsychopharmacol. 7:65-70.

-
- Ling ZD, Robie HC, Tong CW and Carvey PM (1999) Both the antioxidant and D3 agonists actions of pramipexole mediate its neuroprotective actions in mesencephalic cultures. *Journal of Pharmacology and Experimental Therapeutics* **289**:202-210.
- Sramek JJ, Eldon MA, Posvar E, Feng MR, Jhee SS, Hourani J, Sedman AJ, Cutler and NR (1998) Initial safety, tolerability pharmacodynamics, and pharmacokinetics of CI-1007 in patients with schizophrenia. *Psychopharmacology Bulletin* **34**:93-99.
- Witkin JM and Gasior M (1998) Dopamine D3 receptor involvement in the convulsant and lethal effects of cocaine. *Polish Journal of Pharmacology* **50** (suppl):44-45.
- Witkin J, Gasior M, Acra J, Bekman M, Thurkauf A, Yuan J, DeBoer P, Wikstrom H and Dijkstra D (1998) Atypical antipsychotic-like effects of the dopamine D3 receptor agonist (+)-PD 128,907. *European Journal of Pharmacology* **347**:R1-R3.

Selective Serotonin Reuptake Inhibitors

SELECTED COMPOUNDS:

- fluoxetine (Prozac)
- sertraline (Zoloft)
- paroxetine
- WO-09500194

CLINICAL EFFECTS:

Serotonin selective reuptake inhibitors, termed SSRIs, are used therapeutically in the treatment of several psychiatric disorders, including depression and obsessive compulsive disorders. A drawback is that these compounds require repeated administration to produce calming effects.

The SSRIs, including fluoxetine and sertraline, are typically used as oral medication. These compounds are found to be safe and effective and may be used alone or in combination with lithium salts in the treatment of depression.

The onset of effective SSRI drug action is faster when combined with lithium (decreased from 2-3 weeks to less than 1 week) for treatment of depression. New compounds under development (WO 09500194) are being designed with a faster onset of action. Drug development is continuing at a rapid rate in this area due to the large market for the treatment of depression (15 million individuals in North America).

MECHANISM OF ACTION:

The SSRIs act to directly block one type of receptor for the brain serotonin (5-hydroxytryptamine; 5-HT)-containing neurotransmitter system, specifically the serotonin reuptake sites. Once bound tightly to the serotonin reuptake site (sometimes termed uptake site or transporter), these drugs prolong the effects of serotonin already active in the nerve terminal and directly produce an inhibitory effect on the cell bodies synthesizing the neurotransmitter serotonin. Once this selective inhibition is produced, the target projection regions of the serotonergic system produce a compensatory response, which, in turn, produces release of

additional serotonin in brain areas such as the hypothalamus, hippocampus and cortex. It is hypothesized that the increase in the amount of serotonin leads to improved control of behaviors linked this transmitter system, which include aggression, agitation, anxiety, general affect (mood), and sleep, among others. This neurotransmitter system was the first of its kind to be identified with regulation of sleep; enhanced levels of serotonin produced following a large meal containing the precursor tryptophan, which is found in turkey meat, produces the sleepiness associated with ingestion of a Thanksgiving turkey dinner.

In addition to the serotonin reuptake site, there are several serotonin receptor subtypes including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT₃, 5-HT₄, 5-HT₆ and 5-HT₇ receptors, among others. Thus, these receptors confer different pharmacological properties in different areas of the central nervous system.

Other new developments include the targeting of compounds with high selectivity for multiple serotonin receptors and the serotonin reuptake site (e.g. EP-0072294, a 5-HT_{1A} receptor antagonist, 5-HT_{1A} receptor partial agonist and a 5-HT uptake inhibitor). The advantage of these new compounds is the maintenance of selectivity and specificity of the pharmaceutical agent for the serotonin reuptake site combined with a faster onset of drug action due to synergistic drug actions in defined neurons and specific brain regions.

Antidotes to the administration of SSRIs are not available. However, toxic reactions to appropriate doses of this class of drugs are minimal.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

Recent studies have identified that SSRIs reduce the symptoms that accompany personality disorders and modulate a normal personality (Ekselius and Von Knorring, 1999). The clinical efficacy of fluoxetine, sertraline and other SSRIs well accepted in the treatment of major depression and in generalized anxiety disorders. In this regard, treatment with these agents may continue over periods of months and have established a high safety profile for these agents.

These classes of drugs have also long been noted for effects on sleep; indeed, it has been speculated that the improvement in depressive symptoms with SSRI administration may be linked to their ability to improve the onset and quality of sleep in these patients. Studies of young, healthy volunteers conducted under the controlled setting of a sleep laboratory indicate a single-dose administered orally of the SSRI paroxetine acutely enhances the appearance of drowsiness and nausea in a dose-dependent manner (Saletu et al., 1991).

Reports have also indicated treatment of intermittent explosive behavioral disorders with sertraline in adults with impulse control disorders (Feder, 1999). In this regard, the SSRIs are being widely used in the effective management of behavioral disturbances common with dementia (Herrmann and Lanctot, 1998); these behavioral symptoms, which commonly include aggression and agitation, contribute to the premature institutionalization of elderly individuals. While the mechanisms

of the changes in brain function which produce aggression and agitation in these patients is not known, the fact that treatment with SSRIs may reduce the signs and symptoms to a point where the individual can control their behavior serves to underscore a role for the serotonergic neuronal system. Indeed, a role for serotonin in mediating the paradoxical calming effects of psychostimulants in an animal model of hyperactivity has emerged; acute administration of fluoxetine blocks the hyperactivity effects produced by administration of amphetamine or cocaine in a mutant mouse model (Gainetdinov et al., 1999). Administration of sertraline has also been demonstrated to significantly reduce irritability and aggressive outburst in patients with a closed head injury (Kant et al., 1998).

The SSRIs merit further consideration for effective use as a non-lethal technique as based on an extensive review of the medical literature. These drugs are found to be highly effective for numerous behavioral disturbances encountered in situations where a deployment of a non-lethal technique must be considered. This class of pharmaceutical agents also continues to be under intense development by the pharmaceutical industry. It is likely that an SSRI agent can be identified in the near future that will feature a rapid rate of onset.

SSRIs . . . are found to be highly effective for numerous behavioral disturbances encountered in situations where a deployment of a non-lethal technique must be considered.

SELECTED REFERENCES:

- Ekselius, L and Von Knorring, L. (1999) Changes in personality traits during treatment with sertraline or citalopram. *Br. J. Psychiatry* **174**:444-448.
- Feder, R. Treatment of intermittent explosive disorder with sertraline in 3 patients. (1999) *J. Clin. Psychiatry* **60**:195-196.
- Gainetdinove, R. R., Westel, W.C. , Jones, S.R., Levin, E.D., Jaber, M. Caron, M.G. (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* **283**:397-401.
- Herrman, N. and Lanctot, K. L. The management of behavioral disturbances in dementia: The role of serotonergic therapies. (1998) *J drugs* **1**(2):214-220.
- Kant, R., Smith-Seemiller, L., Zeiler, D. Treatment of aggression and irritability after head injury. (1998) *Brain Inj.* **12**:661-666.
- Saletu, B., Frey, R. , Krupka, M., Anderer, P., Grungerger, J., See, W.R. (1991) Sleep laboratory studies on the single-dose effects of serotonin reuptake inhibitors paroxetine and fluoxetine on human sleep and awakening qualities. *Sleep* **14**(5):439-447.

Serotonin 5-HT_{1A} Receptor Agonists

SELECTED COMPOUNDS:

- buspirone (Buspar)
- lesopitron
- alnespirone
- MCK-242
- WAY-100,635
- oleamide

CLINICAL EFFECTS:

Buspirone is a prototypical anxiolytic drug that does not act like a benzodiazepine

due to its actions as a partial agonist at 5-HT_{1A} receptors. Buspirone is highly effective at relieving anxiety without marked sedation. Unlike benzodiazepines, this drug has no hypnotic, anticonvulsant, or muscle relaxant properties nor does it potentiate the CNS depressant effects of conventional sedative-hypnotic drugs, ethanol, or tricyclic antidepressants. Buspirone is typically used in the treatment of generalized anxiety state with its effective use requiring a week or more of treatment. Buspirone produces less psychomotor impairment and is considered to have limited abuse liability as compared to benzodiazepines, such as diazepam. However, tachycardia, palpitations, nervousness, and gastrointestinal distress may occur more frequently with 5HT_{1A} receptor agonists than with benzodiazepines. Buspirone is also clinically useful in assisting in withdrawal from nicotine during smoking cessation programs.

MECHANISM OF ACTION:

There are numerous serotonin receptor subtypes in the central nervous system, including the 5-HT_{1A} receptor. The distribution of this receptor includes a profile of a high density of binding to the serotonin-containing cell bodies located in the midbrain, hippocampus, cortex and amygdala. Thus, pharmaceutical agents which bind to this receptor site with high affinity are situated in areas which control numerous behavioral and physiological functions including cognition, psychosis, feeding/satiety, temperature regulation, anxiety, depression, sleep, pain perception and sexual activity. The 5-HT_{1A} receptor subtype was discovered in 1981 by radioligand binding techniques and cloned in 1988.

The development of 8-OH-DPAT (8-hydroxy-2-dipropyl-amino tetralin) as a selective agonist for the 5-HT_{1A} receptor has led to an understanding of the multiple physiological roles of this receptor as well as its function and distribution in the central nervous system. Central 5-HT_{1A} receptors have been demonstrated to exist in both pre-synaptic (termed somatodendritic autoreceptor) and post-synaptic locations. The physiological activity mediated in the somatodendritic autoreceptor localized on serotonin-containing cell bodies has been established by electrophysiological recording techniques and neurochemical techniques, including microdialysis. Stimulation of the post-synaptic 5-HT_{1A} receptor has been evaluated by a variety of behavioral and physiological measurements and revealed specific roles in temperature regulation and hormone secretion of ACTH (adrenal corticotrophin hormone) which is released in response to stress.

Buspirone, a partial 5-HT_{1A} receptor agonist, has emerged as the first nonbenzodiazepine anxiolytic; thus efficacy of this drug has further confirmed a correlation between the role of serotonin neurotransmission and anxiety. The receptor binding profile of buspirone is high for the 5-HT_{1A} receptor, lower for the 5-HT₇ receptor, and none to low affinity for other 5-HT receptors, noradrenergic, GABA or dopaminergic receptors. Thus, buspirone has highly specific and unique effects on one class of neurotransmitter receptor. Numerous compounds with 5-HT_{1A} receptor agonist profiles similar to buspirone continue to be under active development by the pharmaceutical industry including lesopitron (in Phase II clinical trials), alnespirone (in Phase II clinical trials) and MCK-242 (in Phase II clinical trials).

Several 5-HT_{1A} receptor antagonists, including WAY 100,635, are well characterized and available to selectively block this receptor subtype (Schechter and Kelly, 1997). These selective receptor antagonists are currently being investigated for their use in the treatment of Alzheimer's disease where they are hypothesized to improve cognition by a facilitatory effect on glutamatergic neurotransmission (an excitatory neurotransmitter). The 5-HT_{1A} receptor antagonists are also being investigated for their ability to be useful in the treatment of anxiety disorders by enhancement of 5-HT_{1A} receptor autoreceptor function; this paradoxical effect appears to be a mechanism that induces a more rapid neuroadaptation of the serotonergic system under conditions of chronic 5-HT_{1A} receptor stimulation.

Interestingly, oleamide, a member of a recently recognized family of amidated lipids found in the plasma and cerebrospinal fluid of mammals, has been demonstrated to provide significant neuromodulatory effects at 5-HT_{1A} and 5-HT_{2A} receptors (Boger et al, 1998). Included in this receptor family are two endogenous ligands for the cannabinoid receptor, anandamide and palmitoylethanolamine, which have direct actions on cannabinoid receptor as well as known neuromodulatory properties. Of interest is the demonstration that intraperitoneal administration of oleamide induces sleep and has long-lasting hypothermic effects with distinct neuronal targets in the rat and mouse brain. Oleamide is a compound that potentially may be useful in a clinical setting to enhance the behavioral effects of a 5-HT_{1A} receptor agonist, such as buspirone. Alternatively, oleamide may be useful in producing a sleep-like state when administered alone, although this profile has yet to be fully established in a clinical setting.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

Buspirone is a safe and effective drug for the treatment of anxiety, and should receive consideration for use settings in which a non-lethal technique may be required. The use of a selective 5-HT_{1A} receptor agonist would reduce symptoms of anxiety in an individual or individuals and promote a calmer and more compliant behavioral state. This pharmaceutical agent has direct effects on serotonin receptors in brain regions established as key areas in the regulation of cognition, mood and motor behaviors.

One consideration in the use of buspirone as a non-lethal technique, is the route and duration of treatment required to produce a calm state. Currently, oral medications are required with repeated ingestion over a week or longer period with this compound. Two new formulations are under development including a transdermal (or patch) and a transmucosal route of drug administration (Phase 3 Clinical trial, Bristol-Myers Squibb Co. and Discovery Phase, TheraTech Inc.). Use of a transdermal patch to deliver buspirone may be effective in a prison setting where there may have been a recent anxiety-provoking incident or confrontation and this application warrants further consideration as a specific type of non-lethal technique.

Currently new, more potent second-generation buspirone-like compounds are in clinical trials for use in treatment of anxiety disorders, including lesopitron, MCK-

242, and alnespirone. These compounds, much like buspirone, are both safe and effective in controlling the symptoms of generalized anxiety, which may include marked agitation and restlessness. Alnespirone is also being evaluated for approval in the treatment of aggression as low doses in animal studies were shown to suppress offensive behavior (Drug Report, 1999 Current Drugs; IDDB.com). Significant advantages in bioavailability and side effects may also be found with alnespirone as it is metabolized in a distinctly different manner than buspirone. The cardiovascular effects of alnespirone, however, have not yet been well studied.

Overall, a review of the scientific literature has indicated that buspirone and closely related 5-HT_{1A} receptor agonists are effective in antagonizing isolation-induced (White et al., 1991) and resident intruder (de Boer, et al., 1999) tests of aggression in animals. A role for serotonin neurotransmitter systems continues to be closely linked to aggressive behavior (Moechars et al, 1998). Moreover, the clinical effects of buspirone in selected populations which may be agitated or aggressive, including violent parolees (Cherek, et al., 1999), psychiatrically hospitalized children with symptoms of anxiety and moderately severe aggression (Pfeffer et al., 1997), as well as combat disorders (Hammer et al, 1997), indicate that further research on 5-HT_{1A} receptor agonists is warranted for their consideration as a non-lethal technique.

SELECTED REFERENCES:

- Boger, D.L., Patterson, J.E. and Jin, Q. (1998) Structural requirements for the 5-HT_{2A} and 5-HT_{1A} serotonin receptor potentiation by the biologically active lipid oleamide. *Proc. Natl. Acad. Sci. USA* **95**(8):4102-4107.
- Cherek, D.R., Moeller, F.G., Khan-Dawood, F., Swann, A. and Lane, S.D. (1999) Prolactin response to buspirone was reduced in violent compared to nonviolent parolees. *Psychopharmacology* **142**:144-148.
- DeBoer, S.F., Lesourd, M., Mocaer, E. and Koolhaas, J.M. (1999) Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine_{1A} receptors: A comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. *J. Pharmacol. Exp. Ther.* **288**:1125-1133.
- Hamner, M. Ulmer, H. and Horne, D. (1997) Buspirone potentiation of antidepressants in the treatment of PTSD. *Depress. Anxiety* **5**: 137-139.
- Moechars, D., Gilis, M., Kuiperi, C. Laenen I. And Van Leuven, F. (1998) Aggressive behavior in transgenic mice expressing APP is alleviated by serotonergic drugs. *Neuroreport* **9**:3561-3564.
- Pfeffer, C.R., Jiang, H. and Domeshek, L.J. (1997) Buspirone treatment of psychiatrically hospitalized prepubertal children with symptoms of anxiety and moderately severe aggression. *J. Child. Adolesc. Psychopharmacol.* **7**:145-155.
- Schechter, L.E. and Kelly, M.G. (1997) An overview of 5-HT_{1A} receptor antagonists: historical perspective and therapeutic targets. *Serotonin ID Alert* **2**(7):299-309.
- White, S.M., Kucharik, R.F., Moyer, J.A. (1991) Effects of serotonergic agents on isolation-induced aggression. *Pharm. Biochem. Behav.* **39**:729-736.

Opioid Receptors and Mu Agonists

SELECTED COMPOUNDS:

- morphine
- carfentanil
- Naloxone (antagonist)

CLINICAL EFFECTS:

Although morphine is the prototypic analgesic used to treat moderate to severe pain, an ongoing search for new narcotic agents devoid of side effects predominates current pain research. Possible side effects associated with opioid use include respiratory depression (potentially fatal), miosis (pupillary constriction), sedation and euphoria. A very powerful analgesic, fentanyl, also has a high abuse potential and may be habit forming (and serious life-threatening respiratory depression could occur).

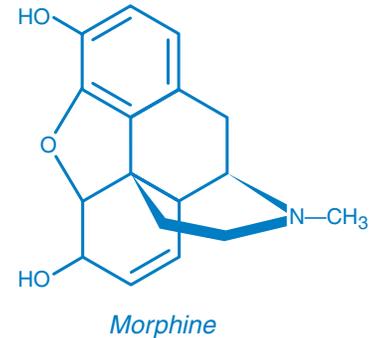
Carfentanil is a narcotic that can be administered via unconventional means (see below). This feature of carfentanil may be very useful in treating non-compliant or unmanageable individuals. New compounds such as *MorphiDex* and *RB-101* are being studied for their ability to alter pain sensation. Carfentanil has a unique utility in the practice of sedating animal populations. This drug has been used successfully to immobilize a variety of large exotic animals and is the only opioid approved in the United States for this purpose. Carfentanil has been administered intramuscularly via dart injection, intravenously and orally.

MECHANISM OF ACTION:

Opioid receptors are classified into three different categories based on their pharmacological profiles. *Mu* (μ), *delta* (δ) and *kappa* (κ) opioid receptors are selectively activated by the endogenous agonists *beta*-endorphin, *met/leu*-enkephalin, and dynorphin, respectively. Recently, a novel opioid receptor, *ORL-1*, has been isolated via cloning techniques. The *ORL-1* receptor and its endogenous ligand *orphanin FQ/nociceptin* are pharmacologically complex, producing both inhibition and potentiation of pain neurotransmission (Pasternak and Letchworth, 1999).

As opioid compounds are capable of producing a wide array of physiological effects, both desired and undesired, the distinct anatomical localization of individual opioid receptor populations becomes particularly important. The distribution of opioid receptors in the central and peripheral nervous systems (CNS and PNS, respectively) correlates with the therapeutic actions and side effects of opiate analgesics. Due to their powerful analgesia-producing properties, *mu* receptors and *mu* receptor-selective agonists have been the primary focus of pain research and management. Each of these effects can be reversed by the administration of the opioid receptor antagonists *naloxone* or *naltrexone*.

These antagonistic agents are therapeutically important due to their ability to



reverse symptoms, particularly respiratory depression, of an opiate overdose. Morphine produces its antinociceptive effects by interacting with *mu* opioid receptors selectively. Fentanyl is a *mu* opioid receptor agonist that has a special usefulness as both a transdermal patch, and a “lollipop” (oral lozenge used to treat surgical pain in children). MorphiDex is a combination of morphine and dextromethorphan (an opioid derivative). MorphiDex shows promise for the treatment of severe pain since it appears to be much more potent than morphine and delivers significantly superior pain relief than similar doses of morphine without increasing side effects.

Carfentanil binds selectively to brain *mu* opioid receptors. Although not yet used in human populations, this drug offers the potential advantage of being administered to non-compliant or violent patients, and requires only indirect contact.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

Morphine is currently the drug of choice for treating moderate to severe pain, but may also produce euphoria, indifference to surroundings, sedation and depressed respiration. Carfentanil has a unique utility in the practice of sedating animal populations. This drug has been used successfully to immobilize a variety of large exotic animals. Carfentanil has been administered intramuscularly via dart injection, intravenously, and orally. Therefore, this drug offers the distinct advantage of being administered to subjects at far distances. Additionally, although not yet used in human populations, this drug offers the potential advantage of being administered to non-compliant or violent patients, requiring only indirect contact. In addition, naloxone and other opioid receptor antagonists are available as an antidote.

DISCUSSION:

Opioids or opiates are narcotic compounds that have very powerful uses as analgesic, sedative, antitussive (anti-cough) and anti-diarrheal agents. The term *opiate* refers to any drug derived from or containing opium. The term *opioid* refers specifically to any non-opium-derived narcotic. For example, the naturally occurring enkephalins and endorphins are considered opioids while morphine is considered an opiate.

Historically, opiates have been used for their mood altering properties. Indeed, the opiate drug *opium* is very unique in that it has been used for hundreds of years, with references to its use dating back to 3000 B.C. It was not until the 1800s that morphine was isolated from opium. Since that time, morphine and other narcotic compounds have been used widely for their analgesic and robust sedative properties. Although morphine is the prototypic analgesic used to treat moderate to severe pain, an ongoing search for new narcotic agents devoid of side effects predominates current pain research.

Discussed below are the various opioid receptors and the specific effects modulated by these receptors. In addition, the latest findings are presented from studies of newly developed analgesic agents that produce their pain-alleviating effects by interacting with the opioid system.

Carfentanil... offers the potential advantage of being administered to non-compliant or violent patients, requiring only indirect contact

As opioid compounds are capable of producing a wide array of physiological effects, both desired and undesired, the distinct anatomical localization of individual opioid receptor populations becomes particularly important. The distribution of opioid receptors in the central and peripheral nervous systems (CNS and PNS, respectively) correlates with the therapeutic actions and side effects of opiate analgesics. For example, in treating pain, the desired effect is antinociception or analgesia, while an unwanted opioid effect (i.e., side effect), in this case, would be constipation. More specifically, μ opioid receptors localized to the spinal cord act to interrupt transmission of pain impulses traveling into the CNS from the periphery (en route to the brain where a noxious stimulus is perceived as being “painful”). As a result, a decreased pain sensation is experienced. However, concurrent stimulation of other opioid receptors localized in the gastrointestinal tract, for instance, may result in constipation (due to a μ/δ receptor-mediated decrease in intestinal peristalsis), an annoying side effect of the narcotic analgesic. Other possible side effects associated with opioid use include respiratory depression (potentially fatal), miosis (pupillary constriction), sedation and euphoria.

Due to their powerful analgesia-producing properties, μ receptors and μ receptor-selective agonists have been the primary focus of pain research and management. As μ receptors are densely populated in CNS structures intricately involved in pain transmission (hippocampus, cerebral cortex, dorsal horn of the spinal cord, periaqueductal grey and thalamus), they are ideally positioned to control pain.

μ opioid receptors are further subdivided into two additional receptor subtypes, μ_1 and μ_2 receptors. The μ_1 receptor subtype produces analgesia when stimulated. Miosis and euphoria are also selective to μ_1 receptor activation while constipation and respiratory depression are associated with μ_2 receptor stimulation (Cherny, 1996). Each of these effects can be reversed by the administration of the opioid receptor antagonists *naloxone* or *naltrexone*. These antagonistic agents are therapeutically important due to their ability to reverse symptoms, particularly respiratory depression, of an opiate overdose.

Morphine produces its antinociceptive effects by interacting with μ opioid receptors selectively (Pasternak, 1988). Morphine is currently the drug of choice for treating moderate to severe pain, but may also produce euphoria, indifference to surroundings, sedation and depressed respiration.

Route of administration is a very important aspect of pharmaceutical development and plays a fundamental role in patient compliancy. For example, fentanyl is a μ opioid receptor agonist that has a special usefulness as both a transdermal patch, and a “lollipop” (oral lozenge used to treat surgical pain in children). Although a very powerful analgesic, fentanyl also has a high abuse potential and may be habit forming (and serious life-threatening respiratory depression could occur). This versatility of drug delivery offers distinct advantages over other pain relievers that are ineffective due to route of administration limitations. Thus, the development of new pain-relieving opiate drugs capable of being administered via several routes

is at the forefront of drug discovery. For example, *carfentanil* is a narcotic that can be administered via unconventional means (see below). This feature of *carfentanil* may be very useful in treating non-compliant or unmanageable individuals.

New compounds such as *MorphiDex* and *RB-101* are being studied for their ability to alter pain sensation. *MorphiDex* is a combination of morphine and dextromethorphan (an opioid derivative). *MorphiDex* shows promise for the treatment of severe pain since it appears to be much more potent than morphine and delivers significantly superior pain relief than similar doses of morphine without increasing side effects. *RB-101* is a non-opioid compound that acts to inhibit the enzymatic breakdown of endogenous opioids, thereby enhancing their analgesic activity. In addition, *RB-101* shows promise in the treatment of opiate withdrawal syndrome.

CARFENTANIL

Although this fentanyl derivative was developed nearly two decades ago, it has gained new interest from the perspective of this report because of the recent pursuit of novel calmatives capable of unconventional administration. *Carfentanil* has a unique utility in the practice of sedating animal populations. This drug has been used successfully to immobilize a variety of large exotic animals (Cornick and Jensen, 1992; Karesh *et al.*, 1998; Kupper *et al.*, 1981; Miller *et al.*, 1996; Ramsay *et al.*, 1995; Seal *et al.*, 1985). It is the only opioid approved in the United States for this purpose.

Carfentanil binds selectively to brain *mu* opioid receptors (Saji *et al.*, 1992; Titeler *et al.*, 1989). Because this opioid has a long duration of action, renarcotization (recurring onset of narcotic effects) may develop 2 to 24 hours after administering an opioid antagonist (Shaw *et al.*, 1995).

Carfentanil has been administered intramuscularly via dart injection, intravenously, and orally (e.g. hand fed or mixed with honey) (Baker and Gatesman, 1985; Ramsay *et al.*, 1995; Seal *et al.*, 1985; Sleeman *et al.*, 1997). Therefore, this drug offers the distinct advantage of being administered to subjects at far distances. Additionally, although not yet used in human populations, this drug offers the potential advantage of being administered to non-compliant or violent patients, in situations requiring only indirect contact. However, it is important to note that prior to the immobilization stage, an initial excitement phase has been reported in animals following administration of *carfentanil* (Raath *et al.*, 1992). Thus, application of this drug to human conditions of belligerence or aggressiveness may require special considerations. These provocative concepts merit future investigation.

SELECTED REFERENCES:

- Baker, J.R. and T. J. Gatesman. (1985) Use of *carfentanil* and a ketamine-xylazine mixture to immobilise wild grey seals (*Halichoerus grypus*). *Vet.Rec.* **116**(8):208-210.
- Cherny, N.I. (1996) Opioid analgesics: Comparative features and prescribing guidelines. *Drugs* **51**, 713-737.
- Cornick, J. L. and J. Jensen. (1992) Anesthetic management of ostriches.

-
- J. Am. Vet. Med. Assoc.* **200**(11):1661-1666.
- Karesh, W. B., M. M. Uhart, E. S. Dierenfeld, W. E. Braselton, A. Torres, C. House, H. Puche, and R. A. Cook. (1998) Health evaluation of free-ranging guanaco (*Lama guanicoe*). *J. Zoo. Wildl. Med.* **29**(2):134-141.
- Kupper, W., N. Drager, D. Mehlitz, and U. Zillmann. (1981) On the immobilization of hartebeest and kob in Upper Volta. *Tropenmed. Parasitol.* **32**(1):58-60.
- Miller, M. W., M. A. Wild, and W. R. Lance. (1996) Efficacy and safety of naltrexone hydrochloride for antagonizing carfentanil citrate immobilization in captive Rocky Mountain elk (*Cervus elaphus nelsoni*). *J. Wildl. Dis.* **32**(2):234-239.
- Pasternak, G. W. (1988) *The Opiate Receptors* (New Jersey, Humana Press).
- Pasternak, G. W. and Letchworth, S. R. (1999) Future opioid analgesics: Targeting the old and the new. *Current Opinion in CPNS Investigational Drugs*, **1**: 54-64.
- Raath, J. P., S. K. Quandt, and J. H. Malan. (1992) Ostrich (*Struthio camelus*) immobilisation using carfentanil and xylazine and reversal with yohimbine and naltrexone. *J. S. Afr. Vet. Assoc.* **63**(4):138-140.
- Ramsay, E. C., J. M. Sleeman, and V. L. Clyde. (1995) Immobilization of black bears (*Ursus americanus*) with orally administered carfentanil citrate. *J. Wildl. Dis.* **31**(3):391-393.
- Saji, H., D. Tsutsumi, Y. Magata, Y. Iida, J. Konishi, and A. Yokoyama. (1992) Preparation and biodistribution in mice of [¹¹C]carfentanil: a radiopharmaceutical for studying brain mu-opioid receptors by positron emission tomography. *Ann. Nucl. Med.* **6** (1):63-67.
- Seal, U. S., S. M. Schmitt, and R. O. Peterson. Carfentanil and xylazine for immobilization of moose (*Alces alces*) on Isle Royale. *J. Wildl. Dis.* **21** (1):48-51, 1985.
- Shaw, M. L., J. W. Carpenter, and D. E. Leith. Complications with the use of carfentanil citrate and xylazine hydrochloride to immobilize domestic horses. *J. Am. Vet. Med. Assoc.* **206** (6):833-836, 1995.
- Sleeman, J. M., W. Carter, T. Tobin, and E. C. Ramsay. Immobilization of domestic goats (*Capra hircus*) using orally administered carfentanil citrate and detomidine hydrochloride. *J. Zoo. Wildl. Med.* **28** (2):158-165, 1997.
- Titeler, M., R. A. Lyon, M. J. Kuhar, J. F. Frost, R. F. Dannals, S. Leonhardt, A. Bullock, L. T. Rydelek, D. L. Price, and R. G. Struble. Mu opiate receptors are selectively labelled by [³H]carfentanil in human and rat brain. *Eur. J. Pharmacol.* **167** (2):221-228, 1989.

Neurolept Anesthetics

Intravenous Anesthesia Induction Agents

SELECTED COMPOUNDS:

- propofol (di-iso-propylphenol)

CLINICAL EFFECTS:

Propofol is a short acting intravenous hypnotic agent, which produces rapid induction of anesthesia. Brief periods of apnea (no breathing) and low blood

pressure may occur with minimal change in heart rate. Reversal occurs spontaneously with minimal hangover or sedative effects (no antidote needed or available). As an intravenous anesthetic agent with minimal side effects, propofol is used extensively on a daily basis for general anesthesia as well as conscious sedation (i.e. giving just enough for a patient to tolerate an unpleasant procedure such as colonoscopy or gastroscopy.)

MECHANISM OF ACTION:

Propofol effects are mediated in the brain and spinal cord by stimulating the GABA receptor. Propofol inhibits neurotransmission by stimulating the chloride channel, which leads to hyperpolarization of the nerve and less likelihood of passing a nerve stimulus.

As a GABA receptor stimulant, propofol is also used extensively with other directly GABA stimulating agents (such as barbituates) and indirect acting GABA agents (such as fentanyl) to produce sedation for prolonged sedation (such as intensive care units).

The synergistic use of agents (where $1+1=3$ versus additive agents where $1+1=2$) is clearly demonstrated in daily practice by propofol. Use of a benzodiazepine (such as midazolam) and a GABA-ergic agent (such as propofol) dramatically decreases the dosage requirements for both agents with enhanced safety profile for both agents.

PROPOSED CONTRIBUTION OF A NON-LETHAL CALMATIVE TECHNIQUE:

Propofol demonstrates a general principle, that the longer a drug is infused (or administered), or the longer the dose given (thereby remaining in the body for a longer time period), the longer the effect of the drug will last. For instance, a short acting (3-5 minute) intravenous anesthetic agent (such as thiopental, "Pentothal") will keep a patient asleep for 3-5 minutes after a single bolus dose, but after a large dose, or prolonged infusion, patients may take 1-3 days to wake up. This is called the context sensitive half-time: the context is the duration of infusion and the half time is the time taken for the drug to decrease by 50%. In contrast, a drug like propofol, when administered for a day or a week, a small or large dose, will only keep the patient asleep for a maximal interval of 30 minutes. Second, no antidote is required or necessary, as metabolism of the drug will occur rapidly and spontaneously.

While no further compounds in the propofol (propyl-phenyl) class are under investigation, the effects of GABA receptor stimulants as a method of producing a calmative effect will be of great interest. The clinical experience of using multiple GABA stimulating agents as well as other synergistic drugs will be directly transferable, as new drugs in all these classes become available.

This topic is recommended for further research and holds great promise for non-lethal applications, and will be a fruitful area for future investigation for non-lethal applications.

...propofol ...a small or large dose, will only keep the patient asleep for a maximal interval of 30 minutes.

Neurolept Anesthetic Combinations

SELECTED COMPOUNDS:

- droperidol and fentanyl combination (Innovar)
- phencyclidines (Ketamine)

CLINICAL EFFECTS:

The combination of these two medications leads to a state of “unawareness” called the neuroleptic state. This differs from the usual anesthetic state where no movement occurs, the patient is well relaxed, and airway obstruction occurs readily. In the neuroleptic state, the patients retain muscle tone, small and large muscle movements occur and airway obstruction is less likely due to the retained muscle tone.

The neuroleptic state is characterized by marked tranquilization and sedation with a state of mental detachment and indifference while reflexes remain essentially intact.

MECHANISM OF ACTION:

The sites of action of droperidol is in the central nervous system (brain and spinal cord) where it interferes with transmission of nerve impulses at dopamine, norepinephrine, serotonin, and GABA synaptic sites.

Neurolept anesthesia was used extensively in the 1970's to avoid the perceived dangerous side effects of inhaled volatile agents. Due to the prolonged duration of action, and the development of new shorter acting agents, the neurolept technique is no longer widely practiced.

PROPOSED CONTRIBUTION AS A NON-LETHAL TECHNIQUE:

Due to the prolonged duration of action, (2-4 hours) and the side effects (alpha blockade with decreases in blood pressure), droperidol is not believed to be of interest as a non-lethal technique. However, the neuroleptic state is of great interest due to the maintenance of muscle tone and reflexes while a state of “unawareness” is produced.

As mentioned, the neuroleptic state has several advantages compared to the usual concept of general anesthesia. For example, droperidol has actions at many types of receptors with a fixed ratio of effects at each receptor type. Based upon general pharmacological principles, it should be possible to develop a mixture of highly specific agents, each coupling to a single receptor type. The concentrations and balance of each agent could be varied to design a mixture, which would reproduce the neuroleptic state without the undesirable side effects. The agents could also be developed (and chosen) to obtain a highly accurate duration of action.

The neurolept anesthetics should be further considered for potential as non-lethal calmative techniques.

Due to the prolonged duration of action... the side effects... droperidol is not believed to be of interest as a non-lethal technique.

Corticotropin-releasing Factor Receptor Antagonists

SELECTED COMPOUNDS:

- CP 154,526 (antagonist)
- NBI 27914 (antagonist)
- CRF-BP (binding protein)

CLINICAL EFFECTS:

Currently in Discovery Phase: no approved clinical use.

MECHANISM OF ACTION:

Corticotropin-Releasing Factor (CRF) is a peptide hormone in humans and mammalian species that regulates basal and stress-induced release of hormones, including ACTH, B-endorphin and other opioid peptides. The physiological effects of CRF are mediated at CRF1 and CRF2 receptors and are closely linked to mood disorders including anxiety and stress. When these receptors are blocked by administration of selective antagonists (CP154,526, NBI 27914) anxiety – related behaviors are alleviated. These CRF antagonists produce calming effects after seizures induced in animal models. Regulation of the binding protein (BP) for CRF also demonstrates a potential therapeutic target for producing a less anxious, calm behavioral state.

The CRF antagonists are a novel approach to producing a calm behavioral state.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

The CRF antagonists are a novel approach to producing a calm behavioral state. These peptides, when combined with new approaches for drug delivery of peptides (see Objective 4) warrant further attention for possible prototype development of a non-lethal calmativative technique.

DISCUSSION:

Corticotropin-releasing factor (CRF), also called corticotropin-releasing hormone (CRH), is a 41- amino acid peptide originally identified from bovine hypothalamus. CRF is highly conserved among species, with human and rat CRF identical to each other and differing only by 7 residues from bovine CRF. In non-mammalian species, two CRF-related peptides, sauvagine and urotensin I, have also been identified from the neurosecretory system of fishes and frogs. Both sauvagine and urotensin I peptides share 50% sequence homology with CRF.

CRF is widely distributed in the brain with highest expression levels in the hypothalamus. It is the major hypophysiotropic factor regulating basal and stress-induced release of adrenocorticotrophic hormone (ACTH), b-endorphin, and other proopiomelanocortin-derived peptides. In addition, CRF is also found in cortical and limbic structures. CRF appears to be both necessary and sufficient for mounting physiological and endocrine responses to stress.

The physiologic effects of CRF are mediated by two G-protein-coupled seven transmembrane-spanning domain receptors, CRF1 and CRF2. Two alternative splice variants for CRF2 have also recently been identified. Localization analysis of CRF1 receptors has identified neocortical, cerebellar, and limbic structures as

the regions which express CRF1 most abundantly, while CRF2 is predominantly found in subcortical areas, most notably the lateral septum and hypothalamus. The distribution of CRF receptors is consistent with the notion that CRF contributes to both emotional behaviors as well as behavioral responses to stress itself.

Behaviorally, intracerebroventricular administration of CRF produces physiologic changes similar to those observed in animal models of stress, including increases in heart rate and blood pressure, alterations in gastrointestinal function, suppression of exploratory behaviors, decreased food intake, and disruption of reproductive behaviors. Importantly, these effects are not observed after systemic administration of CRF nor are they blocked by vagotomy, adrenalectomy, or pretreatment with dexamethasone, suggesting that these effects are mediated by CRF receptors in the central nervous system and do not involve stimulation of the pituitary-adrenal axis. This is further supported by the ability of CRF receptor antagonists to reverse the behavioral effects of exogenously administered CRF.

Recent studies in mouse lines overexpressing CRF have also emphasized the anxiogenic properties of CRF, since these mice behaviorally resemble various animal models of anxiousness. In contrast, CRF knock-out mice exhibited no anxiety-like behaviors; however, this effect may also be due to compensation by other peptidergic and aminergic mechanisms. The importance of each of the CRF receptor subtypes has been examined to determine the impact of CRF in mediating anxiogenic effects. Intracerebroventricular injection of antisense nucleotides to CRF1 receptors in several regions of the brain, but not to CRF2 receptors, resulted in a reduction of anxiety-like behavior when challenged with CRF. These results support the view that CRF1 receptors may be a target for mediating anxiolytic effects associated with CRF.

CRF antagonists have been studied in various animal models of anxiety. Centrally administered CRF fragments or amino acid substitutions of CRF including a peptide termed astressin have been tested in rats. These peptides, which antagonize the action of CRF at CRF1 receptors were found capable of blocking CRF1 receptors and inhibiting ACTH release (Rivier et al., 1999). Other truncated CRF peptides that compete for CRF receptor binding, have also been tested in various models of anxiety in rodents. In some experimental situations truncated CRF peptides attenuate anxiety-like behaviors in rodents; however, the same compounds also induce anxiogenic effects when evaluated under different experimental conditions. The reason for these discrepancies is unclear, but may be related to differing baseline levels of stress. Taken together, these results suggest that CRF1 receptor antagonists may potentially have use as anxiolytics. Therapeutically, peptide antagonists are difficult to administer for a variety of reasons, including rapid degradation and difficulty crossing cellular membranes. For this reason, synthetic CRF receptor antagonists are also under investigation.

Compounds such as CP 154,526 and NBI 27914 inhibit CRF-stimulation of cyclic AMP and CRF-stimulated ACTH release from cultured rat pituitary cells. Furthermore, peripheral administration of these agents to rodents in models of

stress and anxiety attenuates stress-induced elevations of ACTH, suggesting that synthetic CRF1 receptor antagonists may be useful in various neuropsychiatric disorders (McCarthy et al., 1999). In fact, when compared to the atypical anxiolytic buspirone in mouse models of anxiety, CP 154,526 was superior in terms of magnitude of effect and the number of indices of anxiety affected (Griebel et al., 1998). Additionally, CP 154,526 has also been found to possess calming activities in rodent models of "helplessness", a putative model for clinical depression (Mansbach et al., 1997).

In data collected from clinical investigations, there is also evidence for CRF in mediating anxiety-like behaviors. For example, cerebrospinal fluid levels of CRF are reportedly elevated in patients suffering from obsessive compulsive disorder and post-traumatic stress disorder, but not panic disorder. For this reason lipophilic, non-peptide CRF receptor antagonists for use as anxiolytics have been investigated, although currently, there is a dearth of literature supporting the use of these compounds clinically.

CRF-1 receptor antagonists have also been used experimentally for their calming effects on central neurons involved with CRF-induced seizures. In rodent models of CRF-seizures originating in the amygdala, the selective CRF-1 receptor antagonist, NBI 27914, blocked behavioral seizures and prevented epileptic discharges in electroencephalograms (Baram et al., 1997). Taken together, these data support diverse calming roles for CRF-1 receptor antagonists in several aspects of central neuronal activity.

In addition to CRF receptors, another component that regulates CRF function, the CRF binding protein (CRF-BP) may, in the future, also become a target for therapeutic action as a calmative agent. CRF-BP is a protein expressed both in brain, primarily cerebral cortex, amygdala, hypothalamus, hippocampus and pituitary, as well as in plasma. Levels of CRF-BP in plasma determine the amount of "free" CRF available for action at CRF receptors. Currently, pharmacologic agents such as r/h CRF (6-33), a CRF-BP ligand inhibitor, are being used experimentally to study CRF function and physiology. Pharmacologically, r/h CRF (6-33) releases bound CRF from CRF-BP, but r/h CRF (6-33) does not interact with CRF receptors (Heinrichs, 1999). It is conceivable that one approach for future consideration in mediating calming effects in various anxiety states may involve modulation of CRF-BP. Induction of CRF-BP expression or administration of recombinant CRF-BP may be therapeutically useful mechanisms for reducing free circulating levels of CRF, and therefore, may offer benefits clinically if hyperreactivity of CRF neural circuitry or high plasma levels of CRF are contributing factors to anxiety and other stress-related disorders.

SELECTED REFERENCES:

- Baram TZ, Chalmers DT, Chen C, Koutsoukas Y and De Souzaeb (1997) The CRF1 receptor mediates the excitatory actions of corticotropin releasing factor (CRF) in the developing rat brain: In vivo evidence using a novel, selective, non-peptide CRF receptor antagonists. *Brain Research* **770**:89-95.
- Griebel G, Perrault G and Sanger DJ (1998) Characterization of the behavioral

profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone.

Psychopharmacology **138**:55-66.

Heinrichs SC (1999) The role of corticotropin-releasing factor and its receptors in the central nervous system. *Current Opinion in Drug Discovery and Development* **2**:491-496.

Mansbach Rs, Brooks EN and Chen YL (1997) Antidepressant-like effects of CP-154,526, a selective CRF1 receptor antagonist. *European Journal of Pharmacology* **323**:21-26.

McCarthy JR, Heinrichs SC and Grigoriadis DE (1999) Recent advances with the CRF1 receptor: Design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr Pharm Des* **5**:289-315.

Rivier JE, Kirby DA, Lahrchi SL, Corrigan, Vale WW, and Rivier CL (1999) Constrained corticotropin releasing factor antagonists (astressin analogues) with long duration of action in the rat. *Journal of Medicinal Chemistry* **42**:3175-3182.

*The CCKB antagonists
CI-988 and CI-1015
appear to inhibit panic
and induce a calm state. . .*

Cholecystokinin B Receptor Antagonists

SELECTED COMPOUNDS:

- CCK-4
- CI-988 (antagonist)
- CI-1015 (antagonist)

CLINICAL EFFECTS:

Activation of cholecystokinin (CCK) receptors by administration of CCKB agonists produces panic attacks. These effects and other symptoms of anxiety are blocked with administration of selective CCKB antagonists.

MECHANISM OF ACTION:

Cholecystokinin (CCK) is a gut and brain peptide with a variety of actions in the periphery as well as the central nervous system. CCK peptides act at two receptors termed CCK-A and CCK-B. CCK-B receptors are mainly found in the central nervous system. CCK-B receptor agonists induced behavioral changes such as anxiety, disruption of memory, and hyperalgesia. A great deal of evidence implicates CCK involvement in anxiety and panic attacks.

PROPOSED CONTRIBUTION AS A NON-LETHAL CALMATIVE TECHNIQUE:

The CCKB antagonists CI-988 and CI-1015 appear to inhibit panic and induce a calm state via a novel mechanism. Combined with new and improved methods for the delivery of peptide drugs, these compounds warrant further consideration as a non-lethal technique.

DISCUSSION:

Cholecystokinin (CCK) is a gut and brain peptide with a variety of actions in the periphery as well as the central nervous system. CCK was originally isolated three decades ago from porcine gut as a 33 amino acid peptide. Since discovery, several biologically active variants have been found. In the brain, the most abundant

CCK peptide is CCK8, an eight amino acid terminally sulfated peptide. CCK8 has been shown to be involved in numerous physiological functions including feeding behavior, respiratory control, cardiovascular tone, memory processes, nociception, emotional and motivational behaviors.

CCK peptides act at two receptors termed CCK-A and CCK-B. CCK-A receptors are predominantly found in the periphery. In contrast, CCK-B receptors are mainly found in the central nervous system. (Gastrin receptors and CCK- B receptors are identical.). Both CCK-A and CCK-B receptors are members of the seven transmembrane spanning domain G-protein-coupled receptor superfamily. Agonist binding to CCK receptors results in intracellular activation of phospholipase C pathways.

Interest in studying central CCK-B receptors stems largely from studies in which CCK-B receptor agonists induced behavioral changes such as anxiety, disruption of memory, and hyperalgesia. In addition to CCK-8, another bioactive peptide agonist, CCK-4, has been identified in the mammalian brain. The CCK-4 tetrapeptide has a 300-fold higher affinity for CCK-B receptors than CCK-A receptors and has been associated with eliciting anxiogenic actions.

Co-localization of CCK and dopamine in the ventral tegmental area and the mesolimbic pathways of the brain suggest that CCK could act as a neuromodulator of dopaminergic neurotransmission. In the rat, CCK-B mRNA is widely distributed in areas of the cerebral cortex, hippocampus, septum, amygdala, nucleus accumbens, caudate putamen, substantia nigra, and cerebellum. These regions, predominantly mesolimbic areas of the brain, are associated with motivation and reward behaviors. Thus, CCK may also have a role in regulating motivated behaviors, including behaviors of anxiety and fear, through action at CCK-B receptors in the central nervous system.

A great deal of evidence implicates CCK involvement in anxiety and panic attacks. In animals, administration of the selective CCK-B agonist BC 197 results in significant anxiogenic effects. Conversely, in the CCK-B agonist-induced model as well as other models of fear and anxiety, CCK-B receptor antagonists, such as CI-988 have produced anxiolytic actions (Derrien et al., 1994).

Clinical studies have also demonstrated that the endogenous CCK-B receptor agonist CCK-4 causes panic attacks both in patients with histories of such disorders as well as in healthy volunteers. For example, in one placebo-controlled clinical study, administration of CCK-4 induced panic attacks in 71% of the individuals tested (n=12); while injection of saline failed to induce any signs of panic (van Megan et al., 1996). These results demonstrate that anxiety may be mediated at least in part by CCK-4 activity. Since CCK-4 is predominantly expressed in the brain, along with CCK-B receptors, one might extrapolate from this data that CCK-B receptor antagonists may be useful anxiolytic agents.

To test this hypothesis, CI-988, a CCK-B receptor antagonist, was evaluated in a placebo-controlled double-blind three-way cross-over study to determine its

effectiveness in attenuating panic symptoms induced by intravenous administration of CCK-4. Panic attack frequency and a decrease in panic symptoms including chills/hot flashes, chest pain/discomfort and anxiety/fear/apprehension were significantly diminished by CI-988 pretreatment (Bradwejn et al., 1995). These results suggest that CCK-B receptors may mediate the anxiogenic properties of CCK-4 and that CCK-B receptor antagonists may be useful as calming agents.

Although anxiolytic effects were obtained with CI-988, the clinical development of this compound, which structurally is a peptoid derivative, was limited due to poor absorption and efficient hepatic metabolism. Modifications of the chemical structure of CI-988, led to development of CI-1015, for which bioavailability and blood-brain permeability is enhanced. Additionally, newer CCK-B receptor antagonizing compounds have been identified which differ entirely in chemical structure from CI-988 and CI-1015. Examples include the benzodiazepine derivative L-365,260, the ureidoacetamide RP-69758, the diphenylpyrazone LY-288,513, and the asperlicin-related quinazolinones. Chemical modifications of these compounds have resulted in both significant increases in bioavailability and improved aqueous solubility (Noble and Roques, 1999).

Currently, several CCK-B receptor antagonists are being investigated for clinical utility. Two of these compounds are chemically related to benzodiazepines. L-740093, with approximately 10,000-fold more selectivity for CCK-B receptors than CCK-A receptors, is currently in phase I clinical trials for treatment of anxiety disorders. Another agent, L-365260, is currently under evaluation in phase II trials for the treatment of anxiety and benzodiazepine withdrawal symptoms. Another compound, PD-145942, is still in the discovery phase. PD-145942 is under development as potential treatment for anxiety disorders, obesity, pain, and schizophrenia.

Not only may CCK-B receptor antagonists find utility as anxiolytic agents, but various clinical and preclinical studies have demonstrated that CCK-B receptor antagonists may find utility in mediating other calming states as well. Sleep disorders are particularly common in the elderly due to altered awake-sleep rhythms. In aged rats, a CCK-B receptor antagonist GV-150013, was found useful in increasing both REM and non-REM sleep. Additionally, no tolerance was detected after chronic treatment with GV-150013 (Crespi, 1999). Taken together, these results suggest that CCK-B receptor antagonists may also result in improvement in sleep quality.

In addition to synthetically-designed CCK-B receptor antagonists, another approach for CCK-B receptor antagonist drug design may involve modifications in the sequence of CCK peptides themselves. Antagonist properties may be introduced at the peptide level by reducing the length of the sequence or by addition of large, hindering residues. However, because of low biomembrane permeability and rapid degradation, polypeptide therapy is often of limited therapeutic value. Another difficulty with polypeptide therapy is drug delivery across the blood brain barrier. However, a new class of molecules capable of translocating peptides across plasma

membranes in live cells may be useful as peptidic delivery factors for peptides shorter than 30 amino acid residues. Furthermore, transport of small peptides across the blood brain barrier may also be possible via these transport molecules. Thus, the recent discovery of cell-penetrating peptides may open new possibilities in the future with respect to biomedical drug delivery (Lindgren et al., 2000) and CCK-B receptor antagonists may be among the first classes of drugs to benefit from advances in drug delivery biotechnology.

Taken together, recent biomedical advances suggest that not only does a new class of calming agents, CCK-B receptor antagonists, need to be explored further, but also, appropriate delivery methods for getting these compounds to their sites of action must also be considered. More studies are needed to determine not only the effectiveness of CCK-B receptor antagonists as inducers of calmative states, but are also needed to determine the most effective drug designs and delivery approaches.

SELECTED REFERENCES:

- Bradwejn J, Koszycki D, Paradis m, Reece P, Hinton J and Sedman A (1995) Effect of CI-988 on cholecystokinin tetrapeptide-induced panic symptoms in healthy volunteers. *Biological Psychiatry* **38**:742-746.
- Crespi F (1999) Cholecystokinin-B (CCK-B) receptor antagonists improve "aged" sleep: A new class of sleep modulators? *Methods and Findings in Experimental Clinical Pharmacology* **21**:31-38.
- Lindgren M, Hallbrink M, Prochiantz A and Langel U (2000) Cell-penetrating peptides. *Trends in Pharmacological Sciences* **21**: 99-103.
- Noble F and Roques BP (1999) CCK-B receptor: Chemistry, molecular biology, biochemistry and pharmacology. *Progress in Neurobiology* **58**:349-379.
- van Megan HJ, Westenberg HG, Den Boer JA and Kahn RS (1996) The panic-inducing properties of the cholecystokinin tetrapeptide CCK4 in patients with panic disorder. *European Neuropsychopharmacology* **6**:187-194.

Recommendations

Continuing Improvements in Drug Delivery

The controlled delivery of macromolecular drugs, such as peptides, proteins, oligonucleotides and polysaccharides, remain a key issue in the development of calamative agents as non-lethal techniques. However, while many calmatives can be effectively administered by oral, subcutaneous injection or intravenous routes, the development of improved routes of administration as a non-lethal technique. More convenient, painless drug delivery approaches are needed and the development of these methods is a source of much ongoing research. Briefly, we will outline a few of the most recent innovations in macromolecular drug delivery that have considerable potential in the deployment of non-lethal calmative techniques.

...controlled delivery... remain a key issue in the development of calamative agents as non-lethal techniques.

The pharmaceutical industry is focused on development of new and innovative drugs with improvements in increased potency and specificity while retaining appropriate pharmacokinetic and pharmacodynamic profiles. While many of the new drugs are small molecules, such as peptides, other macromolecules such as oligonucleotides, ribozymes, and charged polysaccharides are much larger. Typically, large macromolecules are administered by injection or infusion to achieve the appropriate dose and therapeutic concentrations. Several innovative approaches are under investigation for improving drug delivery via oral, pulmonary, subcutaneous and transdermal routes.

While oral delivery remains a convenient route of drug administration, the gastrointestinal tract continues to present challenges for delivery of agents that are peptides and proteins. The gastrointestinal tract environment can rapidly degrade many compounds, alter their solubility as well as stability; the transport of large molecules across the intestinal mucosa remains difficult. One approach to address this issue is the use of acylated non-alpha-amino acids as low molecular weight carriers to increase the intestinal absorption of a drug; a carrier-drug complex can be formulated as a liquid and administered orally. A different approach is the use of bioadhesive, bioerodable nanospheres which have been engineered to bind to the intestine and then penetrate into and between cells for drug delivery. Other strategies are focused on improving the delivery of targets to specific cell receptors; this may be less useful in the improved development of calmative agents with actions in the central nervous system.

As an alternative to oral ingestion of agents, the delivery of drugs across the oral buccal mucosa is being investigated (see discussion on the 5-HT_{1A} receptor agonist buspirone). TheraTech Inc. is developing an oral, mucoadhesive bioerodible tablet

that sticks to gum and releases the drug of interest in the opposing buccal tissue. A phenomena of drug delivery by “chewing gum” may well be acceptable to an individual on a voluntary basis and/or useful within specific situations of crowd behavior.

The pulmonary route of drug administration continues to offer the advantage of a rapid, non-invasive method of delivery of peptides and proteins. There is considerable information available on the size of particle aerosols that can be inhaled; considerable research and drug development is ongoing in this area (Daddona, 1999).

The use of injectable subcutaneous depot polymer formulations and implantable devices are under extensive investigation for delivery drug peptides and proteins for sustained periods of time (months). Maintaining chemical stability and retaining biological activity following release over extended time periods continues to be a challenge. The effective use of osmotically-driven titanium implants that can protect the drug of interest and provide sustained release of the agent is ongoing (Phase III clinical trials of a Leucopride implant for palliative treatment of advanced prostate cancer). It is not likely that this route of administration will be suitable for implementation with non-lethal calmatative techniques. However, continued improvements in this area of drug delivery should be closely monitored.

There are currently numerous transdermal patches marketed that are effective and safe in providing a controlled delivery of hydrophobic drug molecules through the outer protective layers of the skin. One approach to improve the delivery of peptides, proteins and larger macromolecules through the skin is the use of low-frequency ultrasound to temporarily disrupt the outer skin layer by cavitation and permit delivery of the drug. The application of absolute alcohol to remove the lipid layer of the skin, termed “tape stripping”, is also another approach to disrupt the skin and enhance subsequent passage of a drug into the blood stream for distribution to the brain. The use of silicon microneedles to pierce the skin and provide drug delivery is also an area of ongoing development.

Overall, improvements in encapsulation and delivery techniques will be applicable to many peptides and proteins that act in the brain to induce a calm state. The active transport process which serve to protect the brain (the blood brain barrier) also impede the delivery of many molecules and, hence, require direct surgical intervention to enter the central nervous system. However, the combined strategies of chemical modifications to improve delivery as well as new approaches for drug delivery will provide for future opportunities.

Innovations in drug delivery will be an important strategy towards identification and improvement of the application of calmatives as non-lethal techniques.

New Improvements with Combinations of Drugs

Synergism is emerging as an important area in the applied use of pharmaceutical agents that are active in the central nervous system. Synergism is more than just the additive effect of one compound added to a second compound; it is the principle that two drugs may ultimately may be more effective and have greater effect than

The pulmonary route of drug administration continues to offer the advantage of a rapid, non-invasive method of delivery of peptides and proteins.

...transdermal patches ...are effective and safe in providing a controlled delivery of hydrophobic drug molecules through the outer protective layers of the skin.

predicted by the application of each agent alone. In the clinical setting of the operating room where anesthetic agents are in constant use, demonstrations are emerging daily that indicate that either the doses of combined agents may be reduced to achieve a maximal effect and/or a wider spectrum of action is achieved with selected combinations of agents. This approach may offer considerable advantages in the design of an ideal non-lethal calmative technique.

Developing Partnerships with the Pharmaceutical Industry

The pharmaceutical and biotechnology industries in the United States and abroad are now often surpassing the traditional academic settings in conducting basic and applied pharmacology research. In addition, it is well known that for every one new compound successfully proceeding from the discovery phase through all phases of clinical trials and on to market, perhaps hundreds, if not thousands, of compounds are discarded or shelved by the pharmaceutical industry. Often an unwanted side effect, such as gastrointestinal distress, will terminate the development of a promising new pharmaceutical compound. However, in the variety of situations in which non-lethal techniques are used, there may be less need to be concerned with unattractive side-effects; indeed, perhaps a calmative may be designed that incorporates a less than desirable side-effect (e.g. headache, nausea) as part of the drug profile. Furthermore, it may be appropriate to develop a working relationship with the pharmaceutical industry to better incorporate their knowledge and expertise in developing a non-lethal calmative technique. Perhaps, the ideal calmative has already been synthesized and is awaiting renewed interest from its manufacturer.

The use of pharmacological agents to produce a calm behavioral state ... is a topic with high relevance to achieving the mission of law enforcement and military communities.

SELECTED REFERENCES:

- Behr, J.P., and Demeneix, B.A. (1998) Gene delivery with polycationic amphiphiles and polymers. *Current Research in Molecular Therapeutics* **1**(1):5-12.
- Daddona, P.E. Recent advances in peptide, protein and macromolecule drug delivery. (2000) *Current Opinion in Drug Discovery & Delivery* **2**(2):168-171.
- Lindgren, M., Hallbrink, M., Prochiantz, A and Langel, U. (2000) Cell-penetrating peptides. *Trends in Pharmacol. Sci.* **247**:99-103.
- Schwartz, J.J. and Zhang, S. (2000) Peptide-mediated cellular delivery. *Current Opinion in Molecular Therapeutics* **2**(2):162-167, 2000.

Closing Comments

The use of pharmacological agents to produce a calm behavioral state, particularly as relevant to management of individuals and/or groups that are agitated, aggressive and/or violent, is a topic with high relevance to achieving the mission of law enforcement and military communities. The extensive review conducted on the medical literature and new developments in the pharmaceutical industry has confirmed the relevance and high potential impact of calmatives as a non-lethal technique. Whether used alone or as an adjuvant to enhance the effectiveness of other types of non-lethal techniques, pharmacological agents can effectively act on central nervous system tissues and produce a less anxious, less aggressive, more tranquil-like behavior and, ultimately, an easier to manage individual. The

wide variety of drug classes and specific agents highlighted in this report serve to underscore that the development and use of non-lethal calmatative techniques is achievable and desirable.

It is recommended that further research be continued regarding calmatives as non-lethal techniques. The classes of agents and the compounds highlighted in-depth should receive additional consideration, including collection of basic and applied research information on their pharmacological profiles as well as data on dose-response curves and duration of action profiles following specific routes of administration. Further research efforts should be directed at identifying the most promising routes of drug delivery that may be effective in the varied settings in which non-lethal techniques are to be deployed. It is also recommended that consideration of partnerships with the pharmaceutical industry be explored in order to achieve the goal of a safe and effective use of calmatives as non-lethal techniques.

This report serves to highlight the importance of developing the use of pharmacological agents, such as calmatives, for use as a non-lethal technique. Several major classes of pharmaceutical agents also merit similar review including 1) drugs of abuse (including selected club drugs) and 2) convulsants. These classes of compounds can effectively impart many of the same qualities that have been identified in this report for the central nervous system depressants highlighted in this report. We recommend that these classes of compounds be evaluated in the near future in order to identify the most promising candidates for use as non-lethal techniques.

...the development and use of non-lethal calmatative techniques is achievable and desirable.

Several major classes of pharmaceutical agents also merit similar review including 1) drugs of abuse (including selected club drugs) and 2) convulsants.