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Nucleus accumbens circuitry mediating analgesia in the normal and tolerant rat

Author
Schmidt, Brian Lee

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Nucleus Accumbens Circuitry Mediating
Analgesia in the Normal and Tolerant Rat

by

Brian Lee Schmidt

DISSERTATION

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DOCTOR OF PHILOSOPHY

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in the

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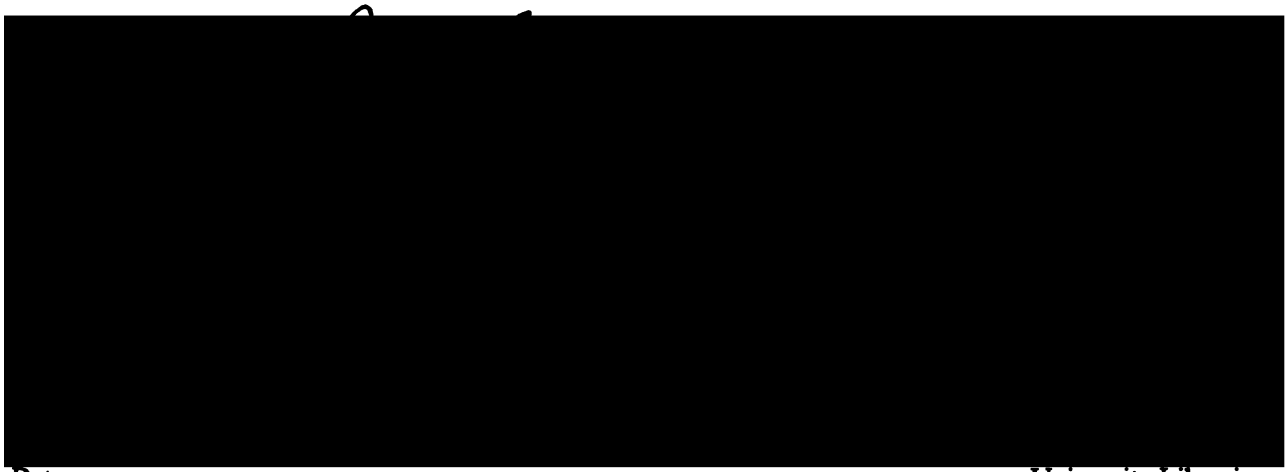

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Dedication

To my father, Cyrus H. Schmidt

Acknowledgements

Many people have contributed directly and indirectly to the development and culmination of this thesis project.

Lei Luo has provided outstanding technical support with many of the experiments and was truly dedicated to the work.

Robert Gear instigated my introduction to the lab and was instrumental in setting up my project. I would like to thank him for the enthusiasm he brings to studying nucleus accumbens and antinociceptive mechanisms; it was that enthusiasm that inspired me to work in the lab. Above and beyond helping me carry out the experiments and analyze the data, Robert was a constant source of encouragement, a joy to collaborate with and the truest of friends. In addition to teaching me all he knew about neuroscience he taught me to live each day deliberately.

I am forever indebted to Jon who made available the formidable resources of his laboratory and the unlimited value of his time. Endlessly enthusiastic and visionary, he gently urged me to do the experiments that he knew would improve the conclusions of this thesis. His constant encouragement, unlimited patience and willingness to let me find my way at my pace have all contributed to my intellectual development over the last couple of years. By his example he has taught me the force of a nonpareil work ethic, the power of maintaining focus on one question at a time and the importance of unyielding respect for those around you. On a practical level, Robert and Jon taught me what to do when the results do not make sense: scrutinize the data with relentless attention to detail, immerse yourself in the literature, refocus your efforts and do another experiment.

Nucleus Accumbens Circuitry Mediating Analgesia in the Normal and Tolerant Rat

by Brian Lee Schmidt

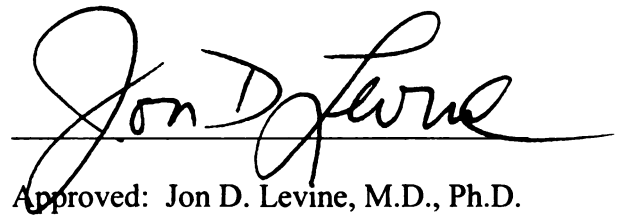
Abstract

The aim of this thesis was to analyze the role of nucleus accumbens nicotinic and opioid receptors in opioid- and pain-induced antinociception. A secondary aim was to assess adaptations in the nucleus accumbens receptors in the mediation of antinociception during chronic morphine and nicotine treatment and following their withdrawal. Receptor-selective antagonists and agonists were microinjected into nucleus accumbens to determine the contribution of individual receptors to nociceptive modulation. The jaw-opening reflex (JOR) was used to measure nociceptive responses in the rat. Intra-nucleus accumbens injection of μ- or δ-opioid receptor antagonists (i.e., Cys^2,Tyr^3,Orn^5,Pen^7amide (CTOP) or naltrindole, respectively), but not κ-opioid receptors (*nor*-binaltorphimine), blocked capsaicin-induced antinociception. Simultaneous intra-accumbens injection of the μ- and δ-opioid agonists ([D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and D-$Pen^{2,5}$-enkephalin (DPDPE), respectively) produced antinociception. The antinociceptive effects of capsaicin and the DAMGO/DPDPE combination were blocked by intra-nucleus accumbens injection of the κ-opioid receptor agonist U69,593. In morphine tolerant rats acute morphine had no

antinociceptive effect; however, intraplantar capsaicin produced antinociception. Pre-injection of CTOP, naltrindole or *nor*-binaltorphimine into nucleus accumbens did not antagonize the antinociceptive effect of capsaicin in morphine tolerant rats; however, flupentixol and mecamylamine blocked capsaicin-induced antinociception. Dopamine release was associated with capsaicin-induced antinociception in naïve and morphine tolerant rats and systemic morphine antinociception in naïve rats. Systemic morphine, on the other hand, did not attenuate the JOR or increase dopamine release in morphine tolerant rats.

Morphine withdrawing rats were tolerant to acute systemic morphine. Capsaicin-induced antinociception was intact and dependent on nucleus accumbens μ-opioid receptors. Intra-accumbens DAMGO resulted in significant antinociception. Intra-accumbens DPDPE did not have an effect by itself and did not enhance the effect of DAMGO. U69,593 continued to antagonize the effect of intra-accumbens DAMGO.

In nicotine naïve rats, intra-accumbens injection of mecamylamine blocked antinociception produced by systemic morphine, intra-accumbens DAMGO/DPDPE, or intraplantar capsaicin. The antinociceptive effect of either morphine or noxious stimulation was unchanged during nicotine tolerance. However, intra-accumbens mecamylamine lost its ability to block antinociception produced by either treatment, and intra-accumbens mecamylamine by itself induced hyperalgesia in nicotine tolerant rats.



Approved: Jon D. Levine, M.D., Ph.D.

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Chapter 1

Introduction

Analgesics, including opiates, cocaine, amphetamine and nicotine have significant abuse potential (Le Magnen et al., 1980). The analgesic and addictive properties of these substances have been speculated to involve overlapping neural mechanisms (Franklin, 1998). The mesolimbic dopaminergic reward pathway and its terminal projection, nucleus accumbens, mediate the addictive properties of these substances of abuse. Increased nucleus accumbens dopaminergic transmission is not only involved with rewarding behavior (Wise, 1989) but also analgesia (Altier and Stewart, 1998). Decreased nucleus accumbens dopaminergic transmission, on the other hand, is associated with the physical signs of withdrawal from nicotine (Fung et al., 1996; Hildebrand et al., 1998; Carboni et al., 2000) and opioids (Diana et al., 1995; Ghosh et al., 1998; Hildebrand, et al., 1998; Diana et al., 1999; Ghosh and Grasing, 1999; Carboni, et al., 2000). The abstinence syndrome that occurs during opioid and nicotine withdrawal is associated with pain-like symptoms (Hughes et al., 1992; Smith et al., 1996; Dunbar and Pulai, 1998; Dunbar et al., 2000); however, the role of nucleus accumbens opioid receptor subtypes and nicotinic receptors in antinociception during tolerance and withdrawal has not been studied. In these studies I analyzed the contribution of these receptors in nucleus accumbens to opioid- and pain-induced antinociception and determined the impact of morphine and nicotine tolerance and withdrawal on nucleus accumbens-mediated antinociception. The results of this project add to our

understanding of supraspinal mechanisms mediating antinociception during the naïve, tolerant and withdrawing states.

The available data suggests that nucleus accumbens involves similar mechanisms for both morphine and nicotine addiction. I hypothesize that nucleus accumbens also involves overlapping and possibly interacting antinociceptive mechanisms for these two substances. Nucleus accumbens mediates the dependence-producing actions of morphine (Wise, 1989) and nicotine (Marshall et al., 1997); in addition, both substances activate nucleus accumbens dopaminergic transmission through similar direct and indirect mechanisms (Johnson and North, 1992; Borg and Taylor, 1997; Marshall, et al., 1997; Kaiser and Wonnacott, 1999; Seppä and Ahtee, 2000). For example, systemic morphine and nicotine both increase dopamine transmission in nucleus accumbens and this increase can be blocked by intra-VTA injections of the appropriate opioid and nicotinic antagonists naloxone and mecamylamine, respectively (Nisell et al., 1994; Pontieri et al., 1996; Tanda and Di Chiara, 1998). Microinjections of morphine or nicotine into either the VTA or nucleus accumbens leads to nucleus accumbens dopamine release (Panagis et al., 1996). Nucleus accumbens dopaminergic transmission is well established to be involved with reinforcement and reward (Wise, 1989) and is also implicated in analgesia (Altier and Stewart, 1999). Support for the hypothesis that morphine and nicotine involve interacting intra-accumbens circuitry comes from data demonstrating that the antagonists of one drug can block the action of the other drug. For example, intra-VTA injections of naloxone block systemic nicotine-induced nucleus accumbens dopamine release (Tanda and Di Chiara, 1998). And, physical signs of nicotine withdrawal can be precipitated by administration of the nicotine antagonist, mecamylamine and the opioid

antagonist, naloxone (Carboni, et al., 2000). These pharmacologic interactions of morphine and nicotine and their respective antagonists in nucleus accumbens suggest that the two substances of abuse might involve and activate common accumbal antinociceptive mechanisms and circuitry.

The nucleus accumbens has a well-established role in modulating nociceptive responses produced by opiates, primarily morphine (Yu and Han, 1989). While μ–, δ– and κ–opioid receptors are present in nucleus accumbens (Svingos et al., 1997; Svingos et al., 1998; Svingos et al., 1999), it is not known which of the receptor subtypes modulate antinociception. Morphine-induced physical dependence is thought to involve adaptations in the three different opioid receptor subtypes in different brain sites, including nucleus accumbens (Trujillo and Akil, 1991; Noble and Cox, 1996; Pan, 1998), and the aversive symptoms that accompany opioid withdrawal are thought to be secondary to dysfunction of endogenous opioid mechanisms (Trujillo and Akil, 1991). To further analyze the nucleus accumbens opioidergic antinociceptive mechanisms, the roles of the opioid receptor subtypes within nucleus accumbens were evaluated during the naïve, morphine tolerant and morphine withdrawing state. Along with evaluating the pharmacologic mechanisms of antinociception, adaptations in the physiologic (pain-induced) mechanisms of antinociception were also evaluated.

Major findings from the present research

The first series of studies was designed to assess the individual roles of the nucleus accumbens μ-, δ-, and κ-opioid receptor subtypes in noxious stimulus-induced antinociception. In addition, to determine whether selective activation of the opioid receptor subtypes was sufficient to produce antinociception the effect of selective opioid

agonism was analyzed. This data is presented in Chapter 2. The μ- and δ- but not κ-opioid receptors were required for capsaicin-induced antinociception. In contrast, intra-nucleus accumbens infusion of the individual selective agonists for μ-, δ-, and κ-receptors failed to produce antinociception. However, simultaneous injection of the μ- and δ-opioid agonists produced antinociception similar to that of intraplantar capsaicin. The antinociceptive effects of capsaicin, as well as that of the μ-/δ-opioid combination, were blocked by intra-accumbens injection of the κ-receptor agonist. Thus, selective activation of individual receptor subtypes is insufficient, but simultaneous activation of intra-nucleus accumbens μ- and δ-opioid receptors is sufficient to induce antinociception, suggesting that co-activation is required. The finding that κ-receptor agonism antagonizes either opioid-induced or noxious stimulus-induced antinociception suggests an anti-analgesic role for nucleus accumbens κ-opioid receptors.

I then tested the hypothesis that chronic morphine treatment produces adaptations in the antinociceptive role of nucleus accumbens μ-, δ- and κ-opioid receptors (Chapter 3). Morphine tolerance was confirmed in these animals by the lack of JOR attenuation following systemic administration of morphine (10 mg/kg). However, the morphine tolerant rats exhibited a robust antinociceptive response to intraplantar capsaicin. Nucleus accumbens opioid receptors were not required for capsaicin-induced antinociception. To confirm the continued role of nucleus accumbens a dopaminergic and nicotinic antagonist were used. Flupentixol, a dopamine antagonist, has been shown to antagonize capsaicin-induced antinociception (Gear et al., 1999). Also, mecamylamine, the nicotinic antagonist, blocks capsaicin-induced antinociception in the

naïve rat (Chapter 5). Both of these antagonists blocked capsaicin-induced antinociception in the morphine tolerant rat. Based on the finding that nucleus accumbens dopamine release mediates both pain-induced (Gear, et al., 1999) and drug-induced antinociception (Altier and Stewart, 1998; Altier and Stewart, 1999) I hypothesized that nucleus accumbens dopamine release might be the substrate maintaining capsaicin-induced antinociception in the morphine tolerant rat. In naïve rats nucleus accumbens dopamine release was associated with jaw-opening reflex (JOR) attenuation following administration of both intraplantar capsaicin and systemic morphine. In morphine tolerant rats JOR attenuation was still associated with an increase in nucleus accumbens dopamine release following intraplantar capsaicin injection; however, systemic morphine did not lead to attenuation of the JOR and did not increase nucleus accumbens dopamine release. These experiments suggest neuroadaptations in nucleus accumbens circuitry mediating pain-induced antinociception following chronic morphine treatment and that capsaicin-induced antinociception is maintained by nucleus accumbens dopaminergic neurotransmission during morphine tolerance.

Because antagonism of nucleus accumbens opioid receptors produces signs of withdrawal (Koob et al., 1989; Stinus et al., 1990) I next analyzed adaptations in the role of nucleus accumbens μ-, δ- and κ-opioid receptors in the mediation of antinociception during morphine withdrawal. Morphine withdrawing rats, like morphine tolerant rats, were resistant to the antinociceptive effects of acute morphine. Also, similar to morphine tolerant rats the withdrawing rats developed a robust antinociceptive response to noxious stimulation. This form of noxious stimulus-induced antinociception was dependent on μ-opioid receptors in nucleus accumbens in morphine withdrawing rats suggesting that the

action of the nucleus accumbens μ-opioid receptor had returned 10 hours following morphine pellet removal. In addition, selective activation of the nucleus accumbens μ-opioid receptor by itself resulted in significant antinociception. Intra-accumbens κ-opioid agonism continued to antagonize the antinociceptive effect of intra-accumbens μ-opioid agonist. Therefore, during the morphine withdrawal state δ-opioid receptor activation appears not to be necessary for nucleus accumbens-mediated antinociception and κ-opioid receptor activation maintains its antianalgesic effect that is observed in naïve rats during spontaneous morphine withdrawal.

Chapter 5 describes the contribution of nucleus accumbens nicotinic receptors to systemic opioid analgesia. In nicotine naïve rats, intra-accumbens injection of the nicotinic receptor antagonist mecamylamine blocked the antinociception produced by three different interventions: systemic morphine, intra-accumbens co-administration of a μ- and a δ-opioid agonist, and noxious stimulation (subdermal capsaicin). The antinociceptive effect of either morphine or noxious stimulation was unchanged during nicotine tolerance; however, intra-accumbens mecamylamine lost its ability to block antinociception produced by either treatment. Intra-accumbens mecamylamine by itself induced significant hyperalgesia in nicotine tolerant rats. These results indicate that nucleus accumbens nicotinic receptors play an important role in both opioid- and noxious stimulus-mediated antinociception in nicotine naïve rats. This role appears to be absent in the nicotine-dependent state, although antagonism of the nicotinic receptor by mecamylamine produced withdrawal hyperalgesia.

Conclusions

The conclusions of this thesis are: 1) nucleus accumbens μ-, δ- and κ-opioid receptors are involved in both pharmacologic and physiologic antinociceptive mechanisms and the opioid receptor subtypes demonstrate both cooperative and opposing antinociceptive interactions, 2) chronic morphine treatment produces adaptations in the antinociceptive roles of the nucleus accumbens opioid receptors, 3) nucleus accumbens nicotinic and opioid antinociceptive mechanisms interact, 4) opioid and nicotinic receptors within nucleus accumbens are required for antinociception; however, nucleus accumbens retains the capacity to mediate significant antinociception during morphine and nicotine tolerance.

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Chapter 2

μ/δ Cooperativity and Opposing κ-Opioid Effects in Nucleus Accumbens-mediated Antinociception in the Rat

Abstract

Noxious peripheral stimulation (e.g., subdermal capsaicin injection in the hindpaw) produces antinociception that is mediated by opioid receptors in nucleus accumbens. The current study used the trigeminal jaw-opening nociceptive reflex responses in the rat to assess the role of intra-accumbens μ-, δ-, and κ-opioid receptors in the antinociceptive effect of noxious stimulation. While intra-accumbens injection of either the μ-receptor selective antagonist Cys^2,Tyr^3,Orn^5,Pen^7amide (CTOP) or the δ-receptor selective antagonist naltrindole blocked capsaicin-induced antinociception, neither the selective μ-agonist [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO, 150 or 300 ng) nor the selective δ-agonist D-$Pen^{2,5}$-enkephalin (DPDPE, 150 or 300 ng) alone induced antinociception. Simultaneous injection of DAMGO and DPDPE (150 ng each) however produced significant antinociception. Capsaicin-induced antinociception was not blocked by the selective κ-receptor antagonist *nor*-binaltorphimine, but was blocked by the κ-*agonist* U69,593. U69,593 also antagonized the antinociceptive effect of the DAMGO/DPDPE combination. Thus, in nucleus accumbens μ- and δ- but not κ-opioid receptors contribute to capsaicin-induced antinociception; selective activation of individual receptor subtypes is insufficient, but coactivation of μ- and δ-opioid receptors

Introduction

Intense noxious (i.e., painful) peripheral stimulation, such as paw immersion in water at ≥ 45°C or intraplantar injection of capsaicin, induces profound heterosegmental antinociception that is similar in magnitude to that produced by high dose morphine 10 mg/kg in the rat (Gear et al., 1999). The involvement of nucleus accumbens endogenous opioids in this antinociceptive effect was demonstrated by the ability of intra-accumbens administration of the nonselective opioid antagonist naloxone to block noxious stimulus-induced antinociception. These findings suggest the existence of a nociceptive control circuit that directly or indirectly conveys signals arising in the periphery to activate antinociceptive mechanisms in nucleus accumbens.

Since opioids constitute a major class of clinically used analgesic medications, it is important to understand the role of intra-accumbens opioid receptors in pain-induced as well as opioid-induced analgesia. While all three subtypes of opioid receptors (i.e., μ, δ, and κ) are present in nucleus accumbens (Svingos et al., 1997; Svingos et al., 1998; Svingos et al., 1999), it is not known which receptor subtypes play a role in pain modulation. In the current study I used receptor-selective antagonists and agonists to determine which opioid receptor subtypes mediate the nociceptive control circuit and also to evaluate their individual roles in opioid-induced analgesia.

Materials and Methods

Animals

Experiments were performed on 280 – 380 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA). These animals were housed in groups of two under a 12 hr light/dark cycle (lights on at 7:00 A.M.) in the University of California San Francisco, animal care facility. Food and water were available *ad libitum*. Experimental protocols were approved by the University of California San Francisco Committee on Animal Research and conformed to NIH guidelines for use of animals in research.

Nociceptive assay

Changes in nociception were measured as attenuation (i.e., antinociception) or enhancement (i.e., hyperalgesia) of the trigeminal jaw-opening reflex (JOR) electromyographic (EMG) signal. I chose this assay (Gear and Levine, 1995; Gear, et al., 1999) because it is segmentally remote from the hindpaw where the noxious stimulus is applied, and thus allows separation of heterosegmental from intrasegmental effects that might influence assays such as the paw-withdrawal reflex or the tail flick reflex. Use of the JOR as a nociceptive assay has been reviewed (Mason et al., 1985).

Anesthesia

All experiments were performed in rats anesthetized with an intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma-Aldrich, St. Louis, MO). This method provides a stable JOR EMG signal over the time period required to complete the experiments (Gear and Levine, 1995).

Electrode implantation

To elicit the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, Inc, South San Francisco, CA). A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

JOR electromyogram

At the beginning of each experiment, stimulation current was set at 3 times the threshold for eliciting a JOR. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive jaw-opening reflex EMG signals evoked by stimulating the tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Baseline amplitude was defined as the average of the last 3 data points, recorded at 5 minute intervals, before an experimental intervention. Effects of experimental interventions are expressed as the mean percentage change ± s.e.m. from the baseline for each experimental group, that is, attenuation, as depicted in the figures, represents a negative percentage change in the JOR baseline EMG.

Cannula placement

For nucleus accumbens injections, 23 gauge stainless steel guide cannulae were stereotactically positioned bilaterally and cemented with orthodontic resin (L.D. Caulk

Co., Milford, DE) to allow injections via insertion of a 30 gauge stainless steel injection cannula, which extended beyond the guide cannulae 2 mm, connected to a 2 μl microsyringe (Hamilton, Reno, NV). Injection volumes were 0.5 μl in all experiments and were carried out over a period of 120 seconds; the injection cannula was left in place an additional 30 seconds. The stereotaxic coordinates for nucleus accumbens injections were: (from bregma) 1.3 mm rostral, 7.2 mm ventral, and ± 1.8 mm lateral. Injection sites were verified by histological examination (100 μm sections stained with cresyl violet acetate) and were plotted on coronal sections adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 1986) (Fig. 1).

Drugs and doses

Capsaicin was dissolved in Tween 80 (50%) and ethanol (50%) to an initial concentration of 50 μg/μl and then diluted with 0.9% saline to a concentration of 5 μg/μl; subdermal capsaicin injection volume was 50 μl (250 μg) in all experiments. [D-Ala^2, *N*-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) 150 ng or 300 ng (Johnson et al., 1995; Noel and Gratton, 1995; Zhang and Kelley, 1997), D-Pen^{2,5}-enkephalin (DPDPE) 150 ng or 300 ng (Johnson, et al., 1995; Meyer and McLaurin, 1995; Zhang and Kelley, 1997), and Cys^2,Tyr^3,Orn^5,Pen^7 amide (CTOP) 1 μg (Ableitner and Schulz, 1992; Devine et al., 1993; Badiani et al., 1995) were dissolved in phosphate buffered saline (PBS). U69,593 100 ng (Spanagel and Shoaib, 1994) was dissolved in 45% aqueous 2-hydroxypropyl-β-cyclodextrin. Naltrindole 1 μg (Kelley et al., 1996; Daugé et al., 1999) and *nor*-binaltorphimine dihydrochloride 1.8 μg (Bodnar et al., 1995; Kelley, et al., 1996) were

dissolved in distilled water. All drugs and reagents were obtained from Sigma-Aldrich, St. Louis, MO or from Sigma-RBI, Natick, MA.

Because it has been reported that *nor*-binaltorphimine may not be selective for κ-opioid receptors until several hours after administration, that is, activity at μ-receptors has been reported (Horan et al., 1992; Spanagel and Shoaib, 1994; Wettstein and Grouhel, 1996), intra-accumbens cannulae were placed under pentobarbital anesthesia (50 mg/kg) and *nor*-binaltorphimine was administered one day prior to the experiment. On the day of the experiment, the rats were anesthetized with α-chloralose/urethane and the usual experimental protocols were followed.

Data Analysis

A two-way repeated measures ANOVA with one between subjects factor (i.e., treatment) and one within subjects factor (i.e, time) was used to determine if there were significant ($p \leq 0.05$) differences in antinociceptive responses among the groups. For each ANOVA the Mauchley criterion was used to determine if the assumption of sphericity for the within-subjects effects was met; if the Mauchley criterion was not satisfied, Greenhouse-Geisser adjusted p values are presented. If there was a significant between-subjects main effect of treatment group, post-hoc contrasts, using the Tukey test, were performed to determine the basis of the significant difference.

Results

Opioid receptor selective antagonists

To evaluate the contribution of intra-accumbens opioid receptor subtypes to noxious stimulus-induced antinociception, selective antagonists, except *nor*-binaltorphimine, were administered to nucleus accumbens 10 minutes prior to the administration of intraplantar capsaicin; *nor*-binaltorphimine was administered the day preceding the experiment (see Methods). CTOP (μ-receptor antagonist) and naltrindole (δ-receptor antagonist) each blocked capsaicin-induced antinociception, but *nor*-binaltorphimine (κ-receptor antagonist) did not significantly affect the capsaicin-induced antinociception (Fig. 2, Table 1). These findings indicate that μ- and δ-opioid receptors in the nucleus accumbens are necessary for noxious stimuli to induce antinociception and that κ-opioid receptors are not involved.

Intra-accumbens μ- and δ-receptor selective agonists

To determine if activation of opioid receptors in nucleus accumbens is sufficient to produce antinociception, receptor selective agonists were administered into nucleus accumbens either alone or in combination. While neither DAMGO (μ-agonist, 150 ng) nor DPDPE (δ-agonist, 150 ng) injected alone into nucleus accumbens affected the JOR, a combination of these doses of DAMGO and DPDPE induced significant antinociception (Fig. 3, Table 1). To test for the possibility that a higher dose of either agonist (i.e., equivalent to the total amount of opioid used in the combination) could induce antinociception, DAMGO (300 ng, n = 12) or DPDPE (300 ng, n = 12) was injected alone into nucleus accumbens. Neither agonist injected alone, at the higher dose,

induced significant antinociception compared to the combination (data not plotted, see Table 1 for statistics).

To test for the possibility that the DAMGO/DPDPE combination induced antinociception at a site outside nucleus accumbens, offsite injections were performed using the same doses. Injections within nucleus accumbens resulted in significantly greater antinociception than did offsite injections (data not plotted; see Fig. 1 for injection sites; Table 1 for statistics).

Intra-accumbens κ-receptor selective agonists

Intra-accumbens injection of the selective κ-agonist U69,593 alone failed to attenuate the JOR (Fig. 4) indicating that intra-accumbens κ-receptor activation is not sufficient to produce antinociception. Therefore, to determine if U69,593 would enhance the antinociceptive effect of the DAMGO/DPDPE combination, the three agonists combined were administered to nucleus accumbens. In contrast to the effect of μ/δ-receptor agonist combination, the combination of all three agonists failed to produce antinociception (Fig. 4, Table 1) suggesting that κ-receptor activation inhibits the antinociceptive effect of μ/δ-receptor activation.

To determine if κ-receptor activation similarly inhibits noxious stimulus-induced antinociception, U69,593 was administered into nucleus accumbens 10 minutes prior to intraplantar capsaicin administration. Intra-accumbens (i.e., onsite) injection of the selective κ-receptor agonist U69,593, but not extra-accumbens (i.e., offsite) injection, significantly attenuated the antinociceptive effect of capsaicin administration (Fig. 5, Table 1). These results suggest that κ-receptors in nucleus accumbens play an anti-

analgesic role for either noxious stimulus-induced antinociception or μ/δ-opioid-induced antinociception.

To confirm that the effect of U69,593 in the previous experiment was mediated by an action at κ-receptors, intra-accumbens U69,593 was administered 10 minutes prior to intraplantar capsaicin in rats treated the preceding day with intra-accumbens *nor*-binaltorphimine (see Methods). U69,593 failed to block the effect of capsaicin in these rats (Fig. 6, Table 1), indicating that the anti-analgesic effect of U69,593 is mediated by κ-receptor activation.

Discussion

μ / δ-opioid receptor co-activation

In this study I demonstrate that selective antagonists for the μ- and δ-opioid receptors in nucleus accumbens block capsaicin-induced antinociception. These results confirm and extend earlier finding that intra-nucleus accumbens administration of the less selective opioid antagonist naloxone blocked capsaicin-induced antinociception (Gear, et al., 1999). In addition, I found that while intra-nucleus accumbens administration of the selective μ- and δ-opioid agonists DAMGO and DPDPE alone did not affect nociception, the combination of DAMGO and DPDPE produced significant antinociception. Thus, nucleus accumbens mediated opioid antinociception requires co-activation of μ- and δ-opioid receptors.

Antinociception that depends on μ/δ-opioid receptor co-activation has been reported previously (Porreca et al., 1987; Heyman et al., 1989b; Negri et al., 1995). For example, spinal antinociception produced by the highly selective δ-opioid receptor agonist DPDPE, in μ-opioid receptor deficient mice is significantly lower than in wild-type mice (Loh et al., 1998; Matthes et al., 1998) suggesting that in normal mice μ-receptors participate in selective δ-agonist-mediated antinociception. While the mechanism underlying the requirement for μ- and δ-opioid receptor co-activation is unknown, a μ/δ-receptor complex has been proposed (Heyman et al., 1989a; Heyman, et al., 1989b; Porreca et al., 1990; Mattia et al., 1991; Cha et al., 1995), and μ/δ-receptor heterodimerization with cross-modulation and synergistic binding of μ and δ agonists has been recently demonstrated (Gomes et al., 2000). Furthermore, anatomic support for a

μ/δ-receptor complex is found in immunocytochemical studies which show that μ- and δ-opioid receptors in nucleus accumbens are co-localized in dendritic spines with apposing enkephalin-labeled terminals (Svingos, et al., 1997; Svingos, et al., 1998). Taken together, these findings suggest that nucleus accumbens opioid-mediated antinociceptive mechanisms, which are activated by intra-accumbens injection of opioid subtype selective agonists, and intraplantar injection of capsaicin, might depend on activation of both μ- and δ-opioid receptors in the form of μ/δ complexes.

Anti-antinociceptive effects of κ-opioid agonism

While intra-accumbens pretreatment with the selective κ-receptor antagonist *nor*-binaltorphimine had no effect on capsaicin or opioid-induced antinociception, administration of the selective κ-*agonist* U69,593 blocked the antinociceptive effect of both intraplantar capsaicin and intra-accumbens DAMGO/DPDPE. U69,593 had no effect on antinociception when injected alone, and also did not block capsaicin or opioid-mediated antinociception after pretreatment with *nor*-binaltorphimine, indicating that U69,593 exerted its effect by acting at κ-receptors. Thus, while intra-accumbens κ-receptor activation does not itself increase nociceptive responses (i.e., produce hyperalgesia), it does exert a strong opposition to μ/δ-receptor-mediated antinociception, whether induced directly by injection of opioid agonists or indirectly by noxious stimulation.

My finding that κ-opioid receptor activation opposes μ/δ-receptor-mediated antinociception supports the suggestion that κ-inhibition of μ-effects is a general theme that can be observed in a number of systems (reviewed by Pan, 1998), including

morphine induced reward (Bolanos et al., 1996; Kuzmin et al., 1997) and morphine tolerance (Tulunay et al., 1981; Schmauss and Herz, 1987; Ramarao et al., 1988; Fujimoto and Holmes, 1990; Takemori et al., 1992; Hooke et al., 1995; Pan et al., 1997). The cellular actions of κ-receptor activation and μ-receptor activation are similar in that both hyperpolarize cells (Pan, et al., 1997). Therefore, κ-antagonism of μ-effects is thought to result from circuit properties such as differential location of these receptor types on pre- and post-synaptic cells (Pan, 1998). In the case of nucleus accumbens, κ-mediated antagonism of antinociception might be explained by its inhibitory effect on nucleus accumbens dopamine release (Spanagel et al., 1992), which may be important in nucleus accumbens nociceptive modulation mechanisms (Altier and Stewart, 1998). For example, capsaicin-induced antinociception is blocked by intra-accumbens injection of a dopamine receptor antagonist (Gear et al., 1999). Furthermore, μ/δ-opioid receptor activation increases nucleus accumbens dopamine release (Xi et al., 1998; Yoshida et al., 1999) and I have found that intraplantar capsaicin injection also increases accumbens dopamine release (Chapter 3). It is therefore possible that κ-opioid antagonism of the antinociceptive treatments in this study resulted from inhibition of dopamine release in nucleus accumbens.

In summary, these results demonstrate that both noxious stimulus-induced antinociception and opioid-induced antinociception in nucleus accumbens depends on activation of both μ- and δ-opioid receptors. My observations that administration of neither DAMGO nor DPDPE alone induced antinociception are compatible with the existence of the proposed μ/δ-receptor complex. The ability of a selective κ–agonist to inhibit both forms of antinociceptive treatment may be explained by κ-receptor

presynaptic regulation of dopamine release. While nucleus accumbens is well known for its role in mediating the effects of substance abuse (Koob, 1992), the present results contribute to the growing body of evidence that it also plays a key role in pain modulation activated by physiologically relevant stimuli (i.e., pain) as well as by opioid administration. Thus, understanding nucleus accumbens pain modulation mechanisms could potentially shed light on improved strategies for the treatment of pain as well as increase understanding of the neural basis of drug addiction.

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Table 1. Statistical summary.

	ANOVAs				Tukey post hocs	
	Effects	DF	*F*	*p*	Groups	*p*
Fig. 2	Tx	3,31	16.558	<0.001	1 v 2	0.308
	Time	3,93	0.353	0.674	1 v 3	<0.001
	Time × tx	9,93	0.797	0.561	1 v 4	<0.001
					2 v 3	0.014
					2 v 4	<0.001
					3 v 4	0.618
Fig. 3	Tx	2,26	6.744	0.004	1 v 2	0.010
	Time	3,78	1.362	0.265	1 v 3	0.016
	Time × tx	6,78	3.580	0.013	2 v 3	0.925
Fig. 3 *(high dose)*[a]	Tx	2,31	5.947	0.007	1 v 2	0.030
	Time	3,93	1.641	0.204	1 v 3	0.008
	Time × tx	6,93	2.541	0.053	2 v 3	0.876
Fig. 3 *(offsite)*[b]	Tx	1,13	7.991	0.014		
	Time	3,39	1.297	0.287	n/a[c]	
	Time × tx	3,39	3.683	0.053		
Fig. 4	Tx	2,25	11.983	<0.001	1 v 2	<0.001
	Time	3,75	3.624	0.017	1 v 3	0.002
	Time × tx	6,75	1.124	0.357	2 v 3	0.576
Fig. 5	Tx	2,21	10.693	0.001	1 v 2	<0.001
	Time	3,63	6.397	0.001	1 v 3	0.515
	Time × tx	6,63	2.827	0.017	2 v 3	0.033
Fig. 6	Tx	2,21	4.806	0.019	1 v 2	0.029
	Time	3,63	1.854	0.161	1 v 3	0.966
	Time × tx	6,63	0.806	0.569	2 v 3	0.039

The discussion and conclusions of this study are based largely on the main effect of treatment ("Tx") and the Tukey post hoc analyses shown in the extreme right column; however, the main effect of time ("Time") and the time × treatment interaction ("Time × tx") are shown for completeness. The identity of the groups in the post hoc column is indicated by the numbers which are given in each of the respective figures (or below).

[a] The results of the two-way ANOVA comparing the effect of systemic DPDPE + DAMGO (group #1, 150 ng each, n = 11), DAMGO (group #2, 300 ng, n = 11), and

DPDPE (group #3, 300 ng, n = 12). The data for DAMGO + DPDPE are plotted in Fig. 3; the data for the other two groups are not plotted.

[b.] The results of the two-way ANOVA comparing the effect of DAMGO + DPDPE (150 ng each) either onsite or offsite (n = 4). The onsite group is plotted in Fig. 3 as group #1; the offsite data are not plotted. All injection sites are shown in Fig. 1.

[c.] Post hoc analysis was not needed because there were only two groups.

Figure 1.

Location of injections. Filled circles are considered to be within the target area of nucleus accumbens; note that they mostly fall within the area of the core. Open circles designate offsite injections. Numbers refer to the distance in mm rostral to bregma (Paxinos and Watson, 1986).

Figure 1

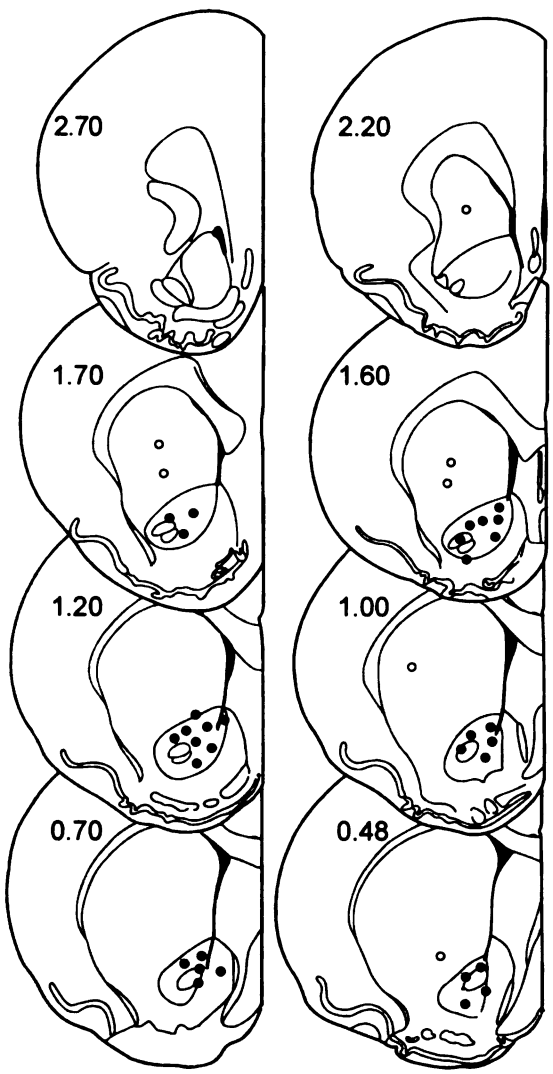


Figure 2.

Effect of intra-accumbens administration of selective opioid antagonists on capsaicin-induced antinociception. Note that both CTOP and naltrindole, but not *nor*-binaltorphimine blocked attenuation of the JOR by capsaicin. In this and subsequent figures antinociception is plotted as per cent attenuation from baseline of the JOR EMG amplitude on the Y-axis (i.e., greater antinociception is represented as higher positive numbers). The group numbers refer to the Tukey post hoc analyses in Table 1; data are plotted as mean ± s.e.m, and the number of rats in each group is shown in parentheses.

Figure 2

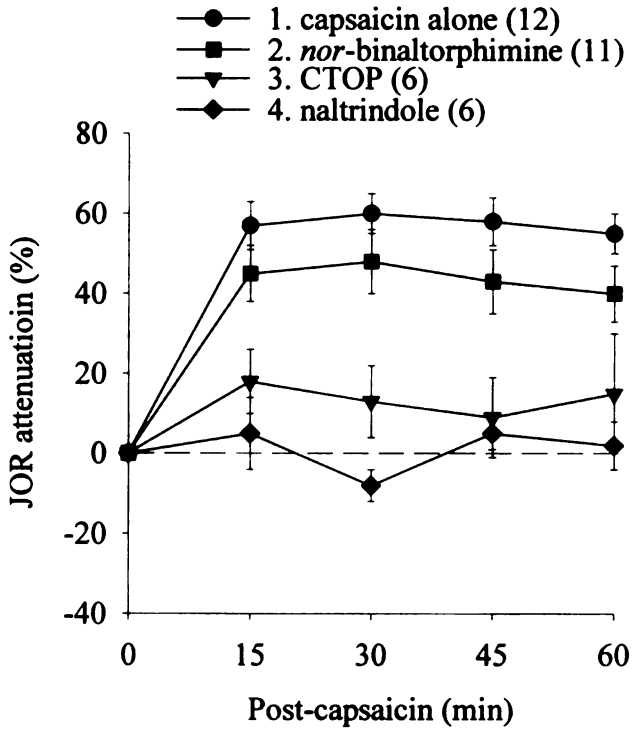

1. capsaicin alone (12)
2. nor-binaltorphimine (11)
3. CTOP (6)
4. naltrindole (6)
JOR attenuatioin (%)
80
60
40
20
0
-20
-40
0
15
30
45
60
Post-capsaicin (min)

Figure 3.

Effect of intra-accumbens administration of selective μ- and δ-opioid agonists alone and in combination. Only the combination of DAMGO plus DPDPE induced significant antinociception. Note that, although the data for the higher doses of DAMGO and DPDPE (300 ng each) are not plotted, the statistics are shown in Table 1.

Figure 3

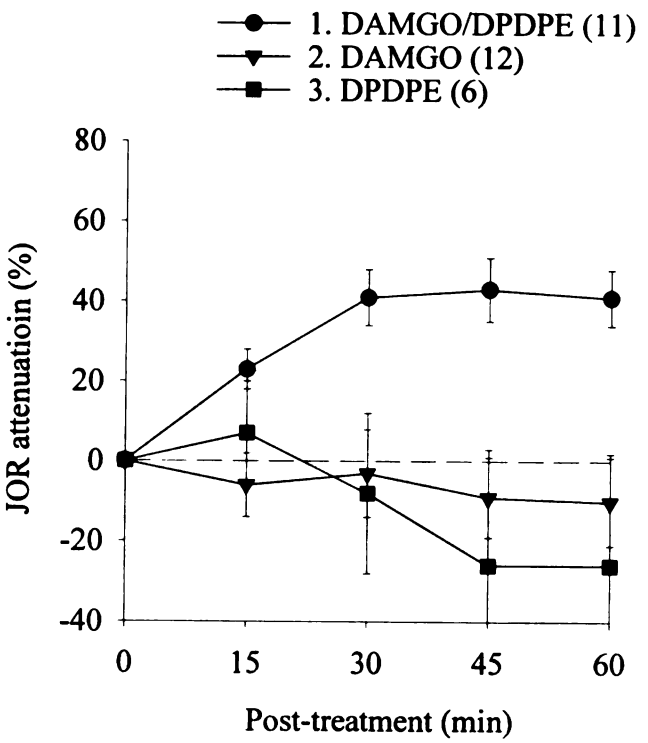

1. DAMGO/DPDPE (11)
2. DAMGO (12)
3. DPDPE (6)
JOR attenuatioin (%)
80
60
40
20
0
-20
-40
0
15
30
45
60
Post-treatment (min)

Figure 4.

Effect of a κ-agonist on the antinociception produced by intra-accumbens administration of μ- and δ-agonists in combination. Although U69,593 had no effect by itself, the addition of U69,593 antagonized the antinociceptive effect of the DAMGO/DPDPE opioid combination.

Figure 4

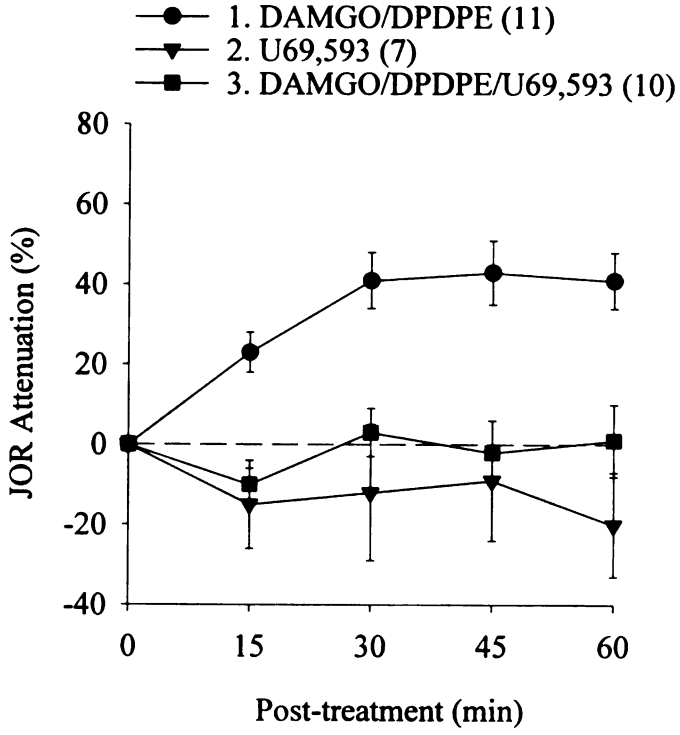

1. DAMGO/DPDPE (11)
2. U69,593 (7)
3. DAMGO/DPDPE/U69,593 (10)
80
60
40
20
0
-20
-40
JOR Attenuation (%)
0
15
30
45
60
Post-treatment (min)

Figure 5.

Effect of a κ-agonist on the antinociception produced by capsaicin. Onsite, but not offsite, intra-accumbens U69,593 injection significantly antagonized the antinociceptive effect of intraplantar capsaicin.

Figure 5

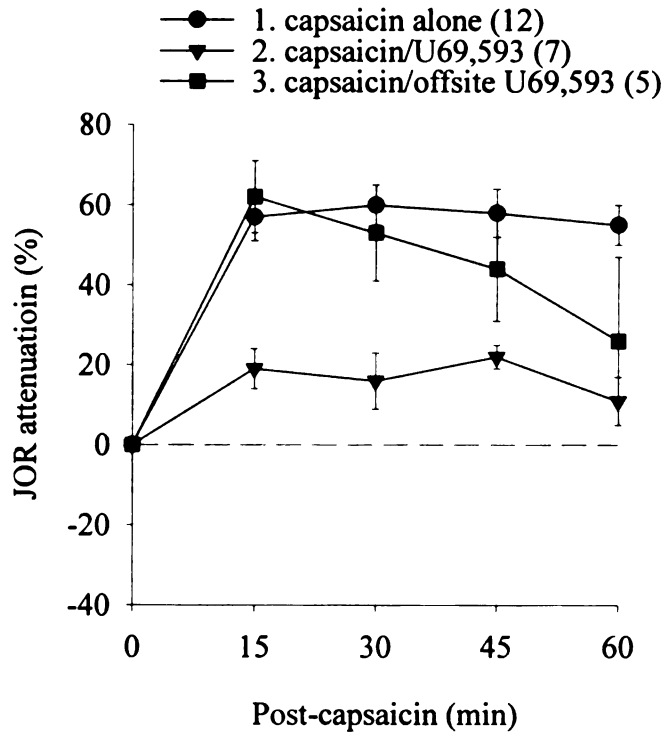
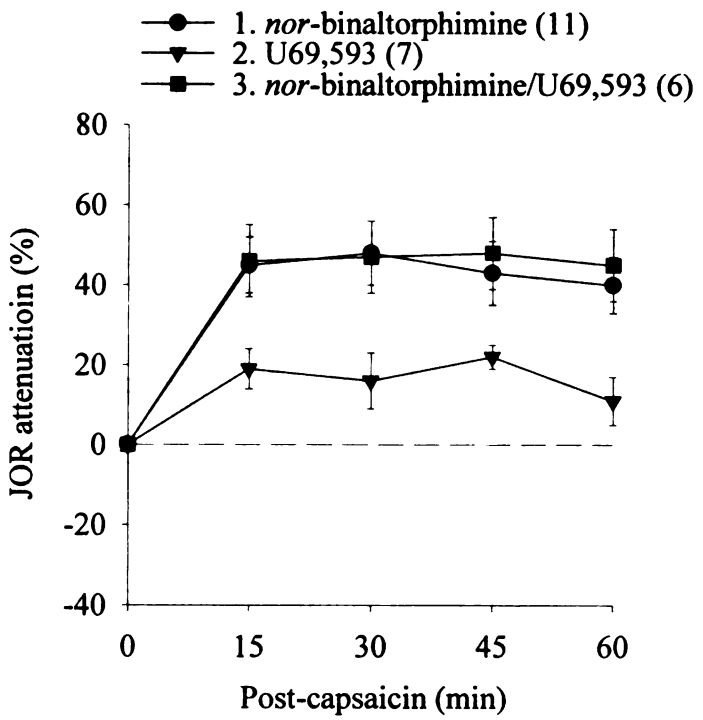

1. capsaicin alone (12)
2. capsaicin/U69,593 (7)
3. capsaicin/offsite U69,593 (5)
JOR attenuatioin (%)
80
60
40
20
0
-20
-40
0
15
30
45
60
Post-capsaicin (min)

Figure 6.

Effect of a κ-antagonist on the antinociception produced by the μ/δ-agonist combination. While pre-treatment with intra-accumbens administration of the κ-antagonist *nor*-binaltorphimine did not itself affect the JOR, this treatment antagonized the ability of the κ-agonist U69,593 to block the antinociceptive effect of capsaicin.

Figure 6


1. nor-binaltorphimine (11)
2. U69,593 (7)
3. nor-binaltorphimine/U69,593 (6)
JOR attenuatioin (%)
80
60
40
20
0
-20
-40
0
15
30
45
60
Post-capsaicin (min)

Chapter 3

Pain-Induced Antinociception in Morphine Tolerant Rats is Mediated by Altered Nucleus Accumbens Circuitry

Abstract

While noxious stimulation induces antinociception that depends on activation of μ- and δ-opioid receptors in nucleus accumbens, chronic morphine treatment did not lead to cross-tolerance to the antinociceptive effects of noxious stimulation. Morphine tolerance did, however, produce changes in the role of nucleus accumbens opioid receptors in mediating noxious stimulus-induced antinociception. In contrast to naïve rats, antagonists for μ- and δ-opioid receptors failed to antagonize the antinociceptive effect of capsaicin. A continued contribution of nucleus accumbens to noxious stimulus-induced antinociception was confirmed by the continued ability of intra-accumbens dopaminergic or nicotinic antagonists to block noxious stimulus-induced antinociception. A contribution of nucleus accumbens dopamine was further supported using brain microdialysis; intra-accumbens dopamine levels increased following capsaicin but not morphine administration in morphine tolerant rats. Thus, unlike opioid analgesia, the opioid-dependent antinociception produced by noxious stimuli is able to escape cross-tolerance to morphine, by an alternative nucleus accumbens mechanism.

Introduction

The clinical use of opioid analgesics is limited by the development of tolerance and/or dependence with repeated administration. Tolerance to the analgesic and toxic effects may dissociate over time; therefore, increasing doses of opiates may provide progressively less analgesia with a worsening side effect profile. Nucleus accumbens is a ventral forebrain structure that mediates both opioid- and pain-induced analgesia and displays differential desensitization of the opioid receptor (Noble and Cox, 1996; Altier and Stewart, 1998; Altier and Stewart, 1999; Gear et al., 1999). Opioid receptors in nucleus accumbens have an important role in nociceptive modulation. Intra-accumbens administration of agonists that act at μ- and δ-receptors, for example morphine (Yu and Han, 1989), as well as a combination of the μ-opioid agonist [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and the δ-opioid agonist D-Pen^{2,5}-enkephalin (DPDPE), induces antinociception (Chapter 2). The antinociception that results from noxious stimulation also depends on μ– and δ–opioid receptors in nucleus accumbens (Chapter 2). The goal of the current study was to determine if morphine tolerance produces cross-tolerance to noxious stimulus-induced antinociception. Since dopamine and nicotinic receptors in nucleus accumbens are important in antinociception (Altier and Stewart, 1999; Seppä and Ahtee, 2000), including noxious stimulus-induced antinociception (Gear, et al, 1999; Schmidt et al, in press), and dopaminergic transmission in nucleus accumbens is disrupted following opiate treatment (Johnson and Glick, 1993; Ghosh and Grasing, 1999), a second goal of this study was to evaluate the response of nucleus accumbens dopamine levels to noxious stimulation in naïve and morphine tolerant rats,

Materials and Methods

Animals

Experiments were performed on 280 – 380 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA). These animals were housed in groups of two under a 12 hr light/dark cycle (lights on at 7:00 A.M.) in the University of California San Francisco, animal care facility. Food and water were available *ad libitum*. Experimental protocols were approved by the University of California San Francisco Committee on Animal Research.

Nociceptive assay

Changes in nociception were measured as attenuation (i.e., antinociception) or enhancement (i.e., hyperalgesia) of the trigeminal jaw-opening reflex (JOR) electromyographic (EMG) signal. I chose this assay (Gear and Levine, 1995; Gear, et al., 1999) in this and previous studies because it is segmentally remote from the hindpaw where the noxious stimulus is applied, thus allowing separation of heterosegmental effects from any intrasegmental effects that might influence assays such as the paw-withdrawal reflex or the tail flick reflex. Use of the JOR as a nociceptive assay has been reviewed (Mason et al., 1985).

Morphine Tolerance Protocol

Morphine tolerance was induced by subcutaneous implantation of 2 morphine base pellets (75 mg, National Institute on Drug Abuse)(Gold et al., 1994). The antinociceptive action of 2 morphine pellets, as measured by tail flick latency, returns to baseline value by 12 to 36 hours (Yoburn et al., 1985; Gold, et al., 1994). Implantation

of pellets occurred under isoflurane anesthesia (Abbott Laboratories, Chicago, IL). Experiments were performed 72 hours following pellet implantation.

Anesthesia

All experiments were performed in rats anesthetized with an intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma-Aldrich, St. Louis, MO). This method provides a stable JOR EMG signal over the time period required to complete the experiments (Gear and Levine, 1995).

Electrode implantation

To elicit the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, Inc, South San Francisco, CA). A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

JOR electromyogram

At the beginning of each experiment, stimulation current was set at 3 times the threshold for eliciting a JOR. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive jaw-opening reflex EMG signals evoked by stimulating the tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Baseline amplitude was defined as the average of the last 3 data points, recorded at 5 minute intervals, before

an experimental intervention. Effects of experimental interventions are expressed as the mean percentage change ± s.e.m. from the baseline for each experimental group, that is, attenuation, as depicted in the figures, represents a negative percentage change in the JOR baseline EMG.

Cannula placement

For nucleus accumbens injections, 23 gauge stainless steel guide cannulae were stereotactically positioned bilaterally and cemented with orthodontic resin (L.D. Caulk Co., Milford, DE) to allow injections via insertion of a 30 gauge stainless steel injection cannula, which extended beyond the guide cannulae 2 mm, connected to a 2 μl microsyringe (Hamilton, Reno, NV). Injection volumes were 0.5 μl in all experiments and were carried out over a period of 120 seconds; the injection cannula was left in place an additional 30 seconds. The stereotaxic coordinates for nucleus accumbens injections were: (from bregma) 1.3 mm rostral, 7.2 mm ventral, and ± 1.8 mm laterally. Injection sites were verified by histological examination (100 μm sections stained with cresyl violet acetate) and were plotted on coronal sections adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 1986) (Fig. 1).

In vivo microdialysis

Animals were anesthetized with pentobarbital sodium, 50 mg/kg (Abbott Laboratories, North Chicago, 60064) and placed in the stereotaxic device. A 12 mm 21 gauge guide cannula was stereotactically positioned and cemented with orthodontic resin (L.D. Caulk Co., Milford, DE) into the right nucleus accumbens: (from bregma) 1.3 mm rostral, 7.2 mm ventral, and 1.8 mm laterally (Paxinos and Watson, 1986)). The experiments were performed 72 hours after guide cannulae placement. A CMA/11

microdialysis probe (CMA/Microdialysis AB, Stockholm, Sweden) was placed on the day of experimentation. The rats were connected to the perfusion system for approximately 180 minutes prior to the experimental intervention. The microdialysis probes were perfused with artificial cerebrospinal fluid (148 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 0.85 mM $MgCl_2$). The pH of the artificial CSF was adjusted to 7.4. The flow rate was set at 2 μL/minute with a CMA/102 microdialysis pump (CMA/Microdialysis AB, Stockholm, Sweden). After a 2 hour equilibration period six baseline fractions were collected every 10 minutes. The experimental interventions were then performed and dialysis samples were collected every 10 minutes and analyzed for dopamine using high-performance liquid chromatography (HPLC) (Legault and Wise, 1999; You et al., 1999).

HPLC analysis

Dopamine was measured with HPLC coupled to electrochemical detection. Dopamine was isolated by injecting dialysate samples with a CMA/200 microsampler (CMA/Microdialysis AB, Stockholm, Sweden) through a 150 x 3 mm column (ESA, MD-150, Chelmsford, MA). Dopamine was quantified by an ESA Coulochem II detector and an analytical cell (ESA model 5011) with two electrodes in series: an oxidizing electrode (+220 mV) and a reducing electrode (-60 mV). The mobile phase consisted of 75 mM sodium phosphate, 1.7 mM 1-octanesulfonic acid, 100 μl/l triethylamine, 25 μM EDTA, 10% acetonitrile; the pH was adjusted to 3.0 with phosphoric acid. The flow rate was pumped at a rate of 0.4 ml/min with a Shimadzu LC-10ADVP (Shimadzu Corporation, Kyoto, Japan).

Drugs and doses

Capsaicin was dissolved in Tween 80 (50%) and ethanol (50%) to an initial concentration of 50 μg/μl and was diluted with 0.9% saline to a concentration of 5 μg/μl; subdermal capsaicin injection volume was 50 μl (250 μg) in all experiments. Cys^2,Tyr^3,Orn^5,Pen^7 amide (CTOP) 1 μg (Ableitner and Schulz, 1992; Devine et al., 1993; Badiani et al., 1995) was dissolved in phosphate buffered saline (PBS). Naltrindole 1 μg (Kelley et al., 1996; Daugé et al., 1999) and *nor*-binaltorphimine dihydrochloride 1.8 μg (Bodnar et al., 1995; Kelley, et al., 1996) were dissolved in distilled water. All drugs and reagents were obtained from Sigma-Aldrich, St. Louis, MO or from Sigma-RBI, Natick, MA.

Because it has been reported that *nor*-binaltorphimine may not be selective for κ-opioid receptors until several hours after administration, that is, activity at μ-receptors has been reported (Horan et al., 1992; Spanagel et al., 1994; Wettstein and Grouhel, 1996), intra-nucleus accumbens cannulae were placed under pentobarbital anesthesia and *nor*-binaltorphimine was administered one day prior to the experiment. On the day of the experiment, the rats were anesthetized with α-chloralose/urethane and the usual experimental protocols were followed.

Data Analysis

A two-way repeated measures ANOVA with one between subjects factor (i.e., treatment) and one within subjects factor (i.e, time) was used to determine if there were significant ($p \leq 0.05$) differences in antinociceptive responses among the groups. For each ANOVA the Mauchley criterion was used to determine if the assumption of sphericity for the within-subjects effects was met; if the Mauchley criterion was not

satisfied, Greenhouse-Geisser adjusted p values are presented. If there was a significant between-subjects main effect of treatment group, post-hoc contrasts, using the Tukey test, were performed to determine the basis of the significant difference.

Results

Morphine tolerance

Although the protocol I used to induce tolerance to morphine is well established, I compared the antinociceptive effect of morphine (10 mg/kg) in rats chronically exposed to morphine (see Methods) and previously untreated (i.e., "naïve") rats (Fig. 2, Table 1). Naïve rats demonstrated significant antinociception compared to morphine-tolerant rats, confirming the existence of tolerance to the antinociceptive effects of high dose morphine.

Noxious stimulus-induced antinociception in morphine-tolerant rats

I also compared the antinociceptive effect of subdermally administered capsaicin (250 μg) into the plantar surface of a hindpaw in morphine-tolerant rats and naïve rats. The antinociceptive effect of this treatment was not significantly different in these two groups, indicating that morphine tolerance does not produce cross-tolerance to noxious stimulus-induced antinociception (Fig. 3, Table 1).

Involvement of nucleus accumbens opioid receptors in noxious stimulus-induced antinociception

I previously observed in morphine naïve rats that noxious stimulus-induced antinociception is mediated in nucleus accumbens by both μ- and δ-, but not κ-, opioid receptors (Chapter 2). To determine if this is the case in morphine-tolerant rats, I administered either CTOP or naltrindole, selective antagonists for μ-, and δ-opioid receptors, respectively, to nucleus accumbens 10 minutes prior to the administration of intraplantar capsaicin. The long-lasting selective κ-receptor antagonist *nor*-binaltorphimine was administered the day before the experiment to avoid the non-

selective action that is reported to occur after acute administration (Horan, et al., 1992; Spanagel, et al., 1994; Wettstein and Grouhel, 1996). The antinociceptive effect of capsaicin following these antagonists was not significantly different from its effect when administered alone (Fig. 4, Table 1). Thus, even though noxious stimulus-induced antinociception is unchanged by morphine tolerance, it does not depend on nucleus accumbens opioid receptors, as is the case in morphine naïve rats. Neither CTOP nor naltrindole administered alone into nucleus accumbens (i.e., without subsequent capsaicin administration) affected the JOR (data not shown).

Involvement of nucleus accumbens in noxious stimulus-induced antinociception during morphine tolerance

Lack of participation by nucleus accumbens opioid receptors in noxious stimulus-induced antinociception in morphine-tolerant rats could indicate either that nucleus accumbens itself no longer plays a role in this phenomenon or that intra-accumbens circuits are reorganized to eliminate dependence on opioid receptors. To determine which of these possibilities is the case, I administered the non-selective dopamine receptor antagonist flupentixol or the nicotinic receptor antagonist mecamylamine 10 minutes prior to capsaicin. Similar to my previous observations in morphine naïve rats (Gear, et al, 1999; Chapter 2), each of these antagonists blocked the antinociceptive effect of capsaicin (Fig. 5, Table 1), indicating that, although opioid receptors are no longer involved, nucleus accumbens itself is still an important neural substrate for noxious stimulus-induced antinociception. To control for the possibility that flupentixol or mecamylamine could act outside of nucleus accumbens to block capsaicin-induced

antinociception, offsite injections were performed. Offsite injections of these agents did not significantly attenuate the JOR (data not shown, injection sites shown in Fig. 1).

Noxious stimulation and nucleus accumbens dopamine levels

Since noxious stimulus-induced antinociception depends on intra-accumbens dopamine receptors in either the morphine naïve or the morphine-tolerant state, I measured the effect of capsaicin administration (250 μg) on nucleus accumbens dopamine release using microdialysis. Dialysate samples were analyzed for dopamine concentration with high pressure liquid chromatography (HPLC, see Methods). To correlate the effect of capsaicin on nucleus accumbens dopamine levels with its effect on nociceptive responses, the JOR was measured simultaneously in some experiments. Intra-accumbens dopamine levels increased after capsaicin injection in both groups; although there appeared to be a spike of dopamine in the naïve group at the 20 minute time point (that likely accounts for the significant time × treatment interaction, Table 1), the overall effect of capsaicin, however, on dopamine was not significantly different between the two groups (Fig. 6). Similarly, the antinociceptive effect of capsaicin in these two groups was not significantly different (Table 1), confirming the finding shown in Figure 2. Taken together, these findings support the suggestion that noxious stimulus-induced antinociception induces dopamine release in nucleus accumbens and that this release correlates closely with antinociception.

Systemic morphine and nucleus accumbens dopamine levels

The effect of subcutaneous injection of morphine (10 mg/kg) on nucleus accumbens dopamine release in morphine-tolerant and naïve rats was assessed in experiments parallel to those above with capsaicin. Morphine induced antinociception as

well as increased intra-accumbens dopamine levels in naïve rats, but did not induce either effect in morphine-tolerant rats (Fig. 7, Table 1), thus further supporting the suggestion that dopamine release in nucleus accumbens correlates with the antinociceptive effect.

Discussion

Despite the dependence of pain-induced antinociception on nucleus accumbens opioid receptors in the naïve rat, morphine tolerance does not reduce the antinociception produced by noxious stimuli. The antinociceptive effect following an intraplantar capsaicin injection is the same in naïve and morphine tolerant rats, equivalent to the level of antinociception produced by high-dose (10 mg/kg) systemic morphine. While the level of pain-induced antinociception is unchanged, nucleus accumbens μ- and δ-opioidergic receptors no longer contribute in morphine tolerant rats. These findings indicate that noxious stimulus-induced antinociception switches from opioid dependent in the naïve state to opioid independent in the morphine tolerant state. Such a switch could result either from an intra-accumbens change in the circuitry or by mediation by a circuit that bypasses nucleus accumbens.

My results demonstrating a change in the requirement of nucleus accumbens opioid receptors in noxious stimulus-induced antinociception during the tolerant state are in line with investigations at the cellular and molecular level showing that chronic opiate treatment alters both the level of nucleus accumbens opioid peptides (Trujillo and Akil, 1990; Nylander et al., 1995) and the function of nucleus accumbens opioid receptors (Noble and Cox, 1996). Extensive changes in other neurotransmitter systems including dopamine have also been shown (Johnson and Glick, 1993; Ghosh and Grasing, 1999; Martin et al., 1999). Chronic morphine treatment results in tolerance in nucleus accumbens dopaminergic transmission (Diana et al., 1995), a finding that was confirmed in my study for opioid analgesia. Similar to other studies, I showed that systemic morphine produced an increase in nucleus accumbens dopamine release in naïve rats

(Pothos et al., 1991; Borg and Taylor, 1997). During morphine tolerance I found that systemic morphine no longer generated a dopamine increase. On the other hand, a dopamine increase correlated with capsaicin-induced antinociception in both naïve and morphine tolerant rats. A comparison of the capsaicin-induced dopamine increase in the naïve and morphine tolerant rats further suggests possible morphine-induced adaptations in the dopaminergic system. Despite a significant capsaicin-induced dopamine increase in morphine tolerant rats the dopamine spike at 20 minutes post-capsaicin observed in the naïve rat was not observed. The requirement for nucleus accumbens dopamine in capsaicin-induced antinociception in morphine tolerant rats was confirmed with pre-injection of flupentixol which antagonized the antinociceptive effect.

I also evaluated the involvement of intra-accumbens cholinergic nicotinic receptors which mediate noxious stimulus-induced antinociception in the naïve rat (Chapter 5). Pretreatment with the nicotinic antagonist mecamylamine blocked noxious stimulus-induced antinociception in morphine tolerant rats, indicating that chronic exposure to morphine induces a change in nucleus accumbens antinociceptive mechanisms such that opioid receptors are no longer required; however, both dopamine and nicotine are involved.

In summary, I demonstrate that while chronic morphine treatment results in tolerance to morphine antinociception pain-induced antinociception is unchanged. The reliance on nucleus accumbens opioid circuitry is modified while the dependence on nucleus accumbens acetylcholine and dopamine remains. The correlation between the persistent antinociception and nucleus accumbens dopamine release points to dopamine as the key neurotransmitter for production of antinociception in morphine tolerant rats.

These findings suggest that a supra-spinal, dopamine-mediated pain modulation system exists that might be effective in the management of intractable pain in patients tolerant to opioid analgesics.

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Table 1. Statistical summary.

	ANOVAs				Tukey post hocs	
	Effects	DF	*F*	*p*	Groups	*p*
Fig. 2	Tx	1,10	13.832	0.004		
	Time	3,30	20.265	<0.001	n/a*	
	Time × tx	3,30	1.336	0.284		
Fig. 3	Tx	1,20	0.740	0.400		
	Time	3,60	0.944	0.393	n/a*	
	Time × tx	3,60	0.143	0.854		
Fig. 4	Tx	3,32	1.139	0.348		
	Time	3,96	6.040	0.004	n/a*	
	Time × tx	9,96	1.413	0.224		
Fig. 5[c]	Tx	2,20	7.579	0.004	1 v 2	0.017
	Time	3,60	5.143	0.012	1 v 3	0.007
	Time × tx	6,60	0.573	0.673	2 v 3	0.731
Fig. 6 (DA)	Tx	1,12	0.486	0.499		
	Time	5,60	3.379	0.040	n/a*	
	Time × tx	5,60	3.133	0.050		
Fig. 6 (JOR)	Tx	1,7	0.018	0.896		
	Time	5,35	1.528	0.249	n/a*	
	Time × tx	5,35	0.095	0.192		
Fig. 7 (DA)	Tx	1,9	16.512	0.003		
	Time	5,45	2.195	0.161	n/a*	
	Time × tx	5,45	2.344	0.147		
Fig. 7 (JOR)	Tx	1,9	42.717	<0.001		
	Time	5,45	0.900	0.431	n/a*	
	Time × tx	5,45	0.402	0.693		

The discussion and conclusions of this study are based largely on the main effect of treatment ("Tx") and the Tukey post hoc analyses shown in the extreme right column; however, the main effect of time ("Time") and the time × treatment interaction ("Time × tx") are shown for completeness. The identity of the groups in the post hoc column is indicated by the numbers which are given in each of the respective figures.

* Post hoc analyses were not done because there were only two groups or because there was no significant main effect of treatment (Fig. 4).

Figure 1.

Location of injections. Filled circles are considered to be within the target area of nucleus accumbens; note that they mostly fall within the area of the core. Open circles designate offsite injections. Filled triangles designate microdialysis probe location. Because some injections were mapped to identical locations, there are fewer circles shown than the total number of injections performed.

Figure 1

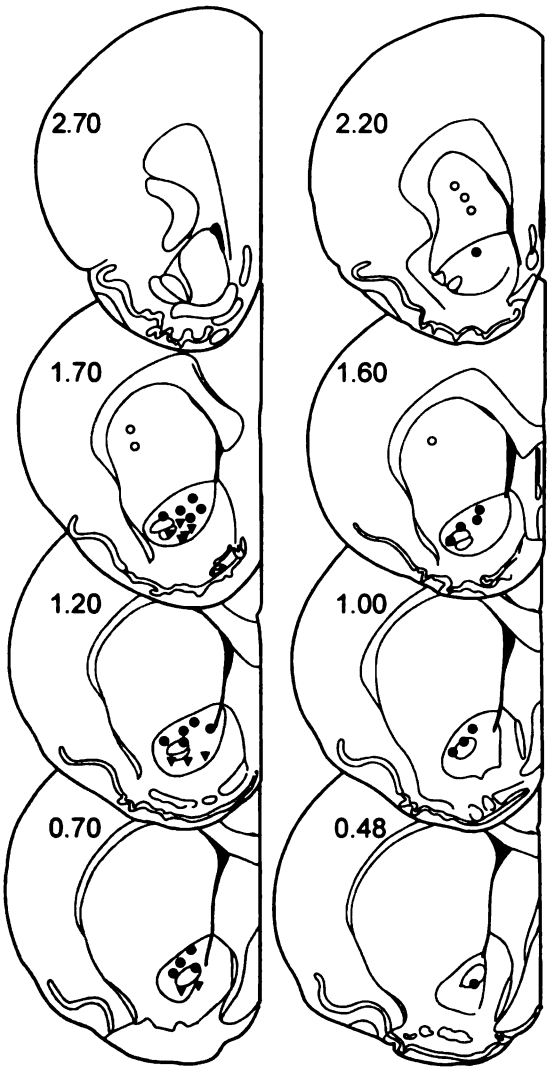


Figure 2.

The antinociceptive effect of subcutaneous morphine administration in morphine-tolerant and naïve rats. The ability of the protocol used to induce morphine tolerance is indicated by the virtually complete disappearance of antinociception following acute morphine administration. In this and subsequent figures antinociception is plotted as per cent attenuation from baseline of the JOR EMG amplitude on the Y-axis (i.e., greater antinociception is represented as higher positive numbers). Baseline JOR recordings were obtained prior to interventions. Time 0 on the X-axis represents the time at which the last (or only) treatment was given for each group. Data are plotted as mean ± s.e.m. Number of rats in each group is shown in parentheses.

Figure 2

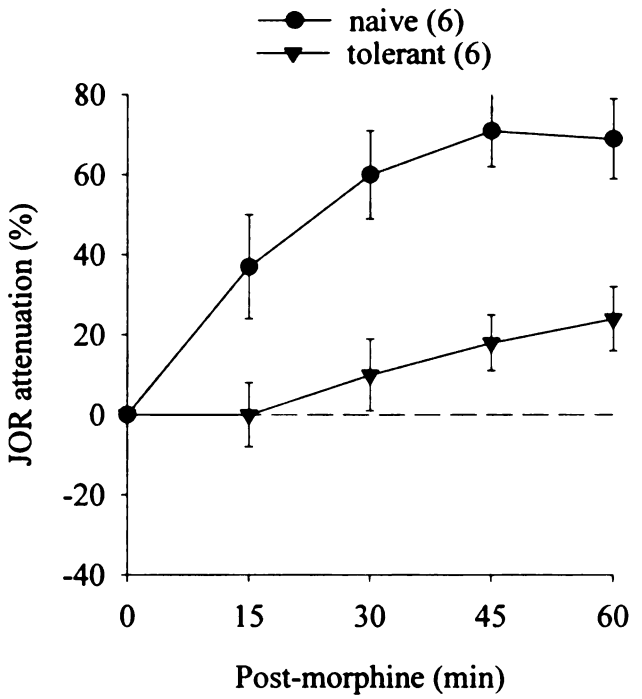

naive (6)
tolerant (6)
80
60
40
20
0
-20
-40
JOR attenuation (%)
0
15
30
45
60
Post-morphine (min)

Figure 3.

The antinociceptive effect of intraplantar capsaicin administration in morphine-tolerant and naïve rats. Absence of cross-tolerance is indicated by the ability of capsaicin to induce a similar degree of antinociception in naïve and morphine-tolerant rats.

Figure 3

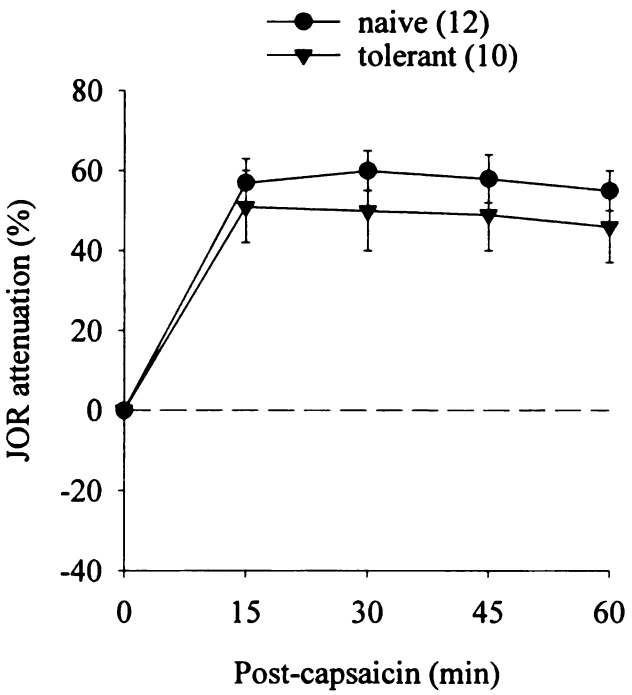

naive (12)
tolerant (10)
80
60
40
20
0
-20
-40
JOR attenuation (%)
0
15
30
45
60
Post-capsaicin (min)

Figure 4.

Effect of selective opioid receptor antagonists administered into nucleus accumbens to block the noxious stimulus-induced antinociception. None of these antagonists significantly reduced capsaicin-induced antinociception, indicating lack of participation of opioid receptors in nucleus accumbens in noxious stimulus-induced antinociception during morphine tolerance.

Figure 4

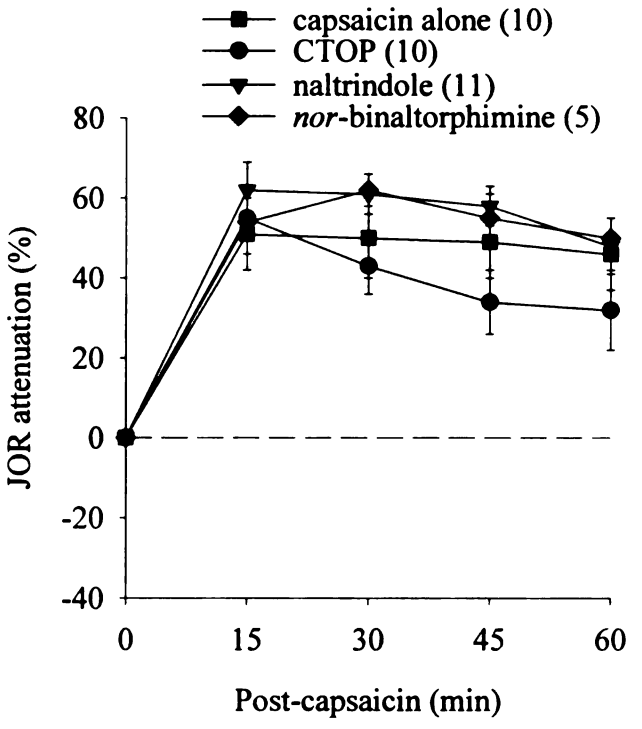

capsaicin alone (10)
CTOP (10)
naltrindole (11)
nor-binaltorphimine (5)
80
60
40
20
0
-20
-40
JOR attenuation (%)
0
15
30
45
60
Post-capsaicin (min)

Figure 5.

Effect of intra-accumbens administration of a nicotinic receptor antagonist or a dopamine receptor antagonist on noxious stimulus-induced antinociception. Either mecamylamine (nicotinic receptor antagonist) or flupentixol (dopamine receptor antagonist) blocked capsaicin-induced antinociception. Group numbers, preceding group names, refer to the Tukey post hoc analyses in Table 1.

Figure 5

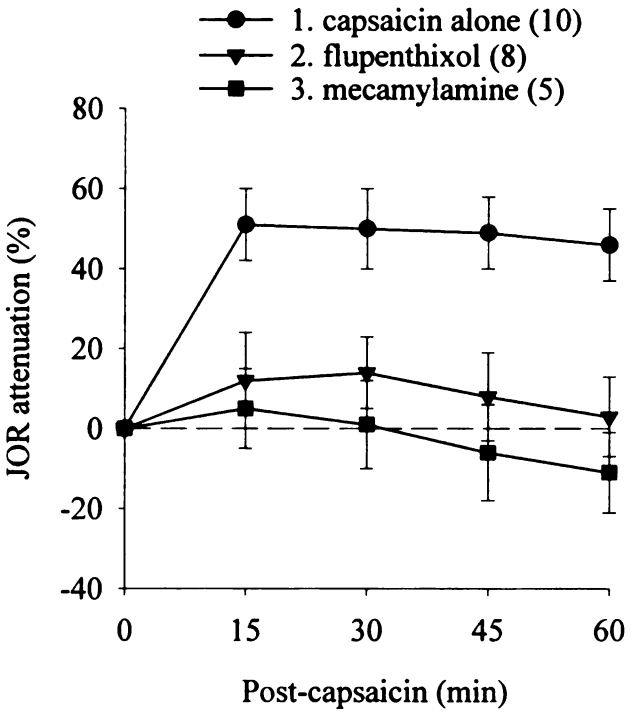

1. capsaicin alone (10)
2. flupenthixol (8)
3. mecamylamine (5)
80
60
40
20
0
-20
-40
JOR attenuation (%)
0
15
30
45
60
Post-capsaicin (min)

Figure 6.

Effect of noxious stimulation on nucleus accumbens dopamine levels in naïve and morphine-tolerant rats. There was no significant difference in dopamine increase or in antinociceptive effect of capsaicin in naïve or morphine-tolerant rats.

Figure 6

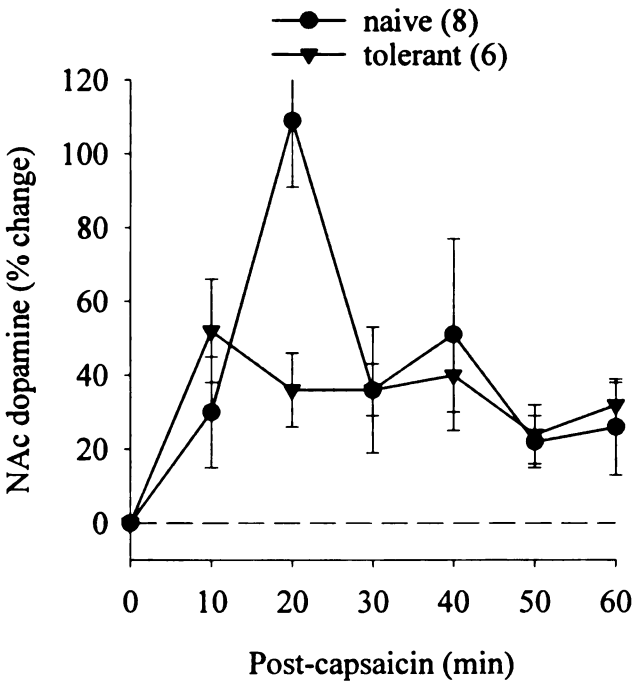
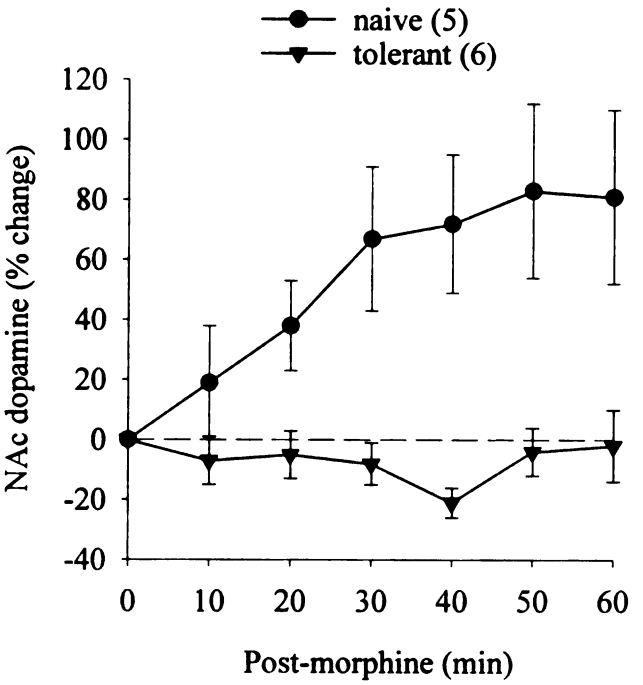

naive (8)
tolerant (6)
NAc dopamine (% change)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Post-capsaicin (min)

Figure 7.

Effect of morphine administration on nucleus accumbens dopamine levels in naïve or morphine-tolerant rats. Morphine induced an increase in dopamine and in antinociception in naïve rats, but neither effect was observed in tolerant rats.

Figure 7


naive (5)
tolerant (6)
120
100
80
60
40
20
0
-20
-40
NAc dopamine (% change)
0
10
20
30
40
50
60
Post-morphine (min)

Chapter 4

Changes in Nucleus Accumbens Opioid Receptor Contributions to Antinociception During Morphine Withdrawal in the Rat

Abstract

I studied adaptations in nucleus accumbens opioidergic mechanisms in the mediation of pain- and opioid-induced antinociception during morphine withdrawal. Rats withdrawing from morphine remained tolerant to the antinociceptive effects of systemic morphine; however, they demonstrated a significant antinociceptive response to noxious stimulation (intraplantar capsaicin). Injection of the μ-opioid receptor antagonist Cys^2,Tyr^3,Orn^5,Pen^7amide (CTOP) alone, but not the δ-opioid antagonist naltrindole, into nucleus accumbens antagonized noxious stimulus-induced antinociception during morphine withdrawal. In naïve rats, intra-accumbens injection of either the μ- or δ-opioid antagonists antagonized noxious stimulus-induced antinociception. While in naïve rats activation of μ- and δ-opioid agonists in nucleus accumbens is required to produce antinociception in morphine-withdrawing rats, intra-accumbens administration of the μ-opioid receptor selective agonist [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) by itself produced significant antinociception. During withdrawal from morphine loss of the nucleus accumbens contribution to morphine analgesia may be due to changes in δ- but not μ-opioid receptors in the nucleus accumbens. As in the opioid naïve state administration of the κ-opioid agonist, U69,593 antagonized the antinociceptive effects of

intra-accumbens DAMGO; however, it no longer antagonized capsaicin-induced antinociception. I conclude that while both nucleus accumbens opioid- and noxious stimulus-induced antinociception display a similar adaptation (attenuation of δ-opioid receptor involvement) during morphine withdrawal, loss of antagonism of noxious stimulus-induced antinociception but not nucleus accumbens opioid antinociception by a κ-opioid agonist suggests underlying mechanistic differences in nucleus accumbens opioid and noxious stimulus-induced antinociception.

Introduction

Nucleus accumbens, a part of the mesolimbic dopaminergic reward pathway, has an important role in the neurologic basis of opiate addiction (Wise, 1989). Nucleus accumbens mediates both the acute reinforcing properties of opiates and the aversive state of opiate withdrawal (Trujillo and Akil, 1991). This ventral forebrain structure is the most sensitive site to the disruptive effects of microinjections of methylnaloxonium on the operant response in morphine dependent rats (Koob et al., 1989b; Stinus et al., 1990). Also, studies utilizing conditioned place aversion have shown that nucleus accumbens is strikingly sensitive to opiate antagonists in opiate dependent rats (Koob et al., 1989a). Avoidance of such withdrawal-induced aversion is hypothesized to maintain drug abuse (Solomon, 1980; Koob, et al., 1989a; Schulteis and Koob, 1996).

Morphine injected into nucleus accumbens can also produce antinociception (Yu and Han, 1989) and it has been shown that nucleus accumbens opioid receptors mediate pain-induced antinociception (Gear and Levine, 1995; Gear et al., 1999). The level of antinociception produced by intraplantar injection of the noxious agent capsaicin is similar in magnitude to that produced by high dose (10 mg/kg) systemic morphine (Gear, et al., 1999).

While the role of nucleus accumbens in mediating certain morphine withdrawal associated behaviors has been studied extensively (Koob, et al., 1989b; Stinus, et al., 1990) nociception has not been evaluated. In the current study I used capsaicin-induced antinociception to evaluate the roles of nucleus accumbens μ-, δ- and κ-opioid receptors in antinociception during morphine withdrawal. Also, microinjections of selective opioid agonists into nucleus accumbens were performed to determine whether there were

Materials and Methods

Animals

Experiments were performed on 280 – 380 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA). These animals were housed in groups of two under a 12 hr light/dark cycle (lights on at 7:00 A.M.) in the University of California San Francisco animal care facility. Food and water were available *ad libitum*. Experimental protocols were approved by the University of California San Francisco Committee on Animal Research and conformed to NIH guidelines for use of animals in research.

Nociceptive assay

Changes in nociception were measured as attenuation (i.e., antinociception) or enhancement (i.e., hyperalgesia) of the trigeminal jaw-opening reflex (JOR) electromyographic (EMG) signal (Mason et al., 1985; Gear and Levine, 1995; Gear, et al., 1999). I chose this assay because it is segmentally remote from the hindpaw where the noxious stimulus is applied, thus allowing separation of heterosegmental effects from any intrasegmental effects that might influence assays such as the paw-withdrawal reflex or the tail flick reflex.

Morphine withdrawal protocol

Morphine tolerance was induced by subcutaneous implantation of 2 morphine base pellets (75 mg, National Institute on Drug Abuse) (Gold et al., 1994). The antinociceptive action of 2 morphine pellets, as measured by tail flick latency, returns to baseline value by 36 hours (Yoburn et al., 1985). Implantation of pellets occurred under isoflurane anesthesia (Abbott Laboratories, Chicago, IL). Removing the morphine pellets 10 hours prior to experimentation induced spontaneous morphine withdrawal. Removal

of the morphine pellets results in rapid biexponential clearance of morphine from the plasma with an initial half-life of 0.74 h, and a terminal half-life of 8.3 h (Yoburn, et al., 1985). Rats tested at 10 hours post pellet removal are in a state of withdrawal and the approximate mean plasma morphine level is only 30 ng/ml (Schulteis et al., 1998).

Anesthesia

All experiments were performed in rats anesthetized with an intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma-Aldrich, St. Louis, MO). This method provides a stable JOR EMG signal over the time period required to complete the experiments (Gear and Levine, 1995).

Electrode implantation

To elicit the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, Inc, South San Francisco, CA). A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

JOR electromyogram

At the beginning of each experiment, stimulation current was set at 3 times the threshold for eliciting a JOR. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive jaw-opening reflex EMG signals evoked by stimulating the

tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Baseline amplitude was defined as the average of the last 3 data points, recorded at 5 minute intervals, before an experimental intervention. Effects of experimental interventions are expressed as the mean percentage change ± s.e.m. from the baseline for each experimental group, that is, attenuation, as depicted in the figures, represents a negative percentage change in the JOR baseline EMG.

Cannula placement

For NAc injections, 23 gauge stainless steel guide cannulae were stereotactically positioned bilaterally and cemented with orthodontic resin (L.D. Caulk Co., Milford, DE) to allow injections via insertion of a 30 gauge stainless steel injection cannula, which extended beyond the guide cannulae 2 mm, connected to a 2 μl microsyringe (Hamilton, Reno, NV). Injection volumes were 0.5 μl in all experiments and were carried out over a period of 120 seconds; the injection cannula was left in place an additional 30 seconds. The stereotaxic coordinates for NAc injections were: (from bregma) 1.3 mm rostral, 7.2 mm ventral, and ± 1.8 mm lateral. Injection sites were verified by histological examination (100 μm sections stained with cresyl violet acetate) and were plotted on coronal sections adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 1986) (Fig. 1).

Drugs and doses

Capsaicin was dissolved in Tween 80 (50%) and ethanol (50%) to an initial concentration of 50 μg/μl and was diluted with 0.9% saline to a concentration of 5 μg/μl; subdermal capsaicin injection volume was 50 μl (250 μg) in all experiments. [D-Ala^2, *N*-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) 150ng or 300ng (Johnson et al., 1995; Noel and

Gratton, 1995; Zhang and Kelley, 1997), D-Pen^{2,5}-enkephalin (DPDPE) 150ng or 300ng (Johnson, et al., 1995; Meyer and McLaurin, 1995; Zhang and Kelley, 1997), and Cys^{2},Tyr^{3},Orn^{5},Pen^{7} amide (CTOP) 1 μg (Ableitner and Schulz, 1992; Devine et al., 1993; Badiani et al., 1995) were dissolved in phosphate buffered saline (PBS). U69,593 100 ng (Spanagel and Shoaib, 1994) was dissolved in 45% aqueous 2-hydroxypropyl-β-cyclodextrin. Naltrindole 1 μg (Kelley et al., 1996; Daugé et al., 1999) and *nor*-binaltorphimine dihydrochloride 1.8 μg (Bodnar et al., 1995; Kelley, et al., 1996) were dissolved in distilled water. All drugs and reagents were obtained from Sigma-Aldrich, St. Louis, MO or from Sigma-RBI, Natick, MA.

Because it has been reported that *nor*-binaltorphimine may not be selective for κ-opioid receptors until several hours after administration, that is, activity at μ-receptors has been reported (Horan et al., 1992; Spanagel et al., 1994; Wettstein and Grouhel, 1996), intra-nucleus accumbens cannulae were placed under pentobarbital anesthesia and *nor*-binaltorphimine was administered one day prior to the experiment. On the day of the experiment, the rats were anesthetized with α-chloralose/urethane and the usual experimental protocols were followed.

Data Analysis

A two-way repeated measures ANOVA with one between subjects factor (i.e., treatment) and one within subjects factor (i.e, time) was used to determine if there were significant ($p \leq 0.05$) differences in antinociceptive responses among the groups. For each ANOVA the Mauchley criterion was used to determine if the assumption of sphericity for the within-subjects effects was met; if the Mauchley criterion was not satisfied, Greenhouse-Geisser adjusted p values are presented. If there was a significant

Results

Morphine withdrawal

Spontaneous morphine withdrawal was induced by removal of the two 75 mg morphine pellets that had been implanted 72 hours earlier to induce tolerance and dependence (Yoburn, et al., 1985; Gold, et al., 1994; Schulteis, et al., 1998). The experiments were performed 10 hours following pellet removal. At 10 hours following pellet removal rats were observed to display the classic signs of morphine withdrawal including wet dog shakes, lacrimation, teeth chattering, piloerection, frequent defecation, and priapism (data not shown) (Maldonado et al., 1992a; Maldonado et al., 1992b).

Antinociceptive effect of systemic morphine administered during morphine withdrawal

The antinociceptive effect of subcutaneously administered morphine (10 mg/kg) in rats undergoing morphine withdrawal or in previously untreated (naïve) rats was compared (Fig. 2, Table 1). Naïve rats demonstrated significant antinociception compared to morphine-withdrawing rats, indicating that tolerance to the antinociceptive effects of high dose morphine remains intact at this stage of withdrawal. This finding is similar to what I previously observed in morphine-tolerant rats (Chapter 3).

Noxious stimulus-induced antinociception in morphine-withdrawing rats

I also compared the antinociceptive effect of subdermally administered capsaicin (250 μg) into the plantar surface of a hindpaw in morphine-withdrawing rats and naïve rats. The antinociceptive effect of this treatment was not significantly different in these two groups, indicating that morphine withdrawal does not modulate noxious stimulus-induced antinociception (Fig. 3, Table 1), a finding that is similar to my previous observation in morphine-tolerant rats (Chapter 3).

Involvement of nucleus accumbens opioid receptors in noxious stimulus-induced antinociception

I previously observed in naïve rats that noxious stimulus-induced antinociception is mediated in nucleus accumbens by both μ- and δ-, but not κ-opioid receptors (Chapter 2). However, morphine tolerance appears to completely eliminate any participation of opioid receptors in noxious stimulus-induced antinociception, even though the antinociceptive effect of noxious stimulation remains intact (Chapter 3) similar to my current finding (above). Therefore, to test the role of opioid receptors in noxious stimulus-induced antinociception in morphine-withdrawing rats, I administered either CTOP or naltrindole, selective antagonists for μ-, and δ-opioid receptors, respectively, to nucleus accumbens 10 minutes prior to the administration of intraplantar capsaicin. The long-lasting selective κ-receptor antagonist *nor*-binaltorphimine was administered the day before the experiment to avoid the non-selective action that is reported to occur after acute administration (Horan, et al., 1992; Spanagel, et al., 1994; Wettstein and Grouhel, 1996). Although the antinociceptive effect of capsaicin following intra-accumbens administration of either naltrindole or *nor*-binaltorphimine was not significantly different from its effect when administered alone (Fig. 4, Table 1), CTOP completely blocked capsaicin-induced antinociception. These findings indicate that μ-, but not δ- or κ-opioid receptors, are required for noxious stimulus-induced antinociception in the morphine-withdrawing state.

Intra-accumbens opioid agonists

I previously found that intra-accumbens administration of a combination of the μ-receptor agonist DAMGO and the δ-agonist DPDPE is required to induce antinociception

in naïve rats; that is, neither of these agonists administered alone produced antinociception, suggesting that μ/δ-receptor coactivation is required in nucleus accumbens for the induction of antinociception. To determine if activation of intra-accumbens opioid receptors can induce antinociception in morphine-withdrawing rats, I administered DAMGO and DPDPE alone and in combination into nucleus accumbens. DAMGO 150 ng was administered; 30 minutes later 450 ng was administered. The JOR was recorded 15 and 30 minutes after the first injection and similarly after the second injection. The same protocol was followed for DPDPE (150 ng/450 ng) and for the combination of DAMGO/DPDPE (same doses as when given individually). Both the DAMGO/DPDPE combination and DAMGO by itself produced antinociception; DPDPE by itself had no effect on the JOR (Fig. 5, Table 1). There was no significant difference between the effect of DAMGO by itself and the effect of the DAMGO/DPDPE combination, suggesting that DAMGO alone mediated the effect. Thus, similar to my finding in morphine-tolerant rats, μ-receptor agonism in nucleus accumbens can induce antinociception, even in morphine-withdrawing rats.

κ-receptor mediated antianalgesia

I previously found that intra-accumbens administration of the κ-receptor agonist U69,593 inhibits the antinociceptive effects of intra-accumbens administration of μ and δ-receptor agonists in naïve rats and μ-receptor agonists in morphine-tolerant rats. To determine if this is also the case in morphine-withdrawing rats, U69,593 (100 ng) was administered either alone or in combination with DAMGO (450 ng). These groups were compared to the group that received DAMGO (150 ng/450 ng) in the previous experiment (above). Neither the DAMGO/U69,593 combination nor U69,593 by itself

significantly affect the JOR (Fig. 6, Table 1), suggesting that U69,593 antagonized the antinociceptive effect of DAMGO, consistent with my previous findings.

To determine if intra-accumbens κ-agonism blocks noxious stimulus-induced antinociception, as observed in both naïve and tolerant rats, U69,593 (100 ng) was administered 10 minutes prior to capsaicin injection. In contrast to my previous findings, U69,593 had no significant effect on capsaicin-induced antinociception (Fig. 7, Table 1), indicating that κ-agonism does not decrease noxious stimulus-induced antinociception in morphine-withdrawing rats.

Discussion

Capsaicin-induced antinociception during morphine withdrawal

I analyzed the role of nucleus accumbens opioid receptor subtypes in the mediation of pain- and opioid agonist-induced antinociception during morphine withdrawal. I demonstrated that while morphine-withdrawing rats remain tolerant to the acute antinociceptive effect of systemic high-dose morphine, noxious stimulus-induced antinociception remained intact. The antinociceptive effect of intraplantar capsaicin in these spontaneously withdrawing rats was not significantly different from that found in naïve rats, equal to the maximum level of antinociception produced by either intraplantar capsaicin or high-dose systemic morphine in the naïve animal. I have previously shown that morphine tolerant rats also exhibit capsaicin- but not morphine-induced antinociception (Chapter 3). Therefore, although morphine tolerant and withdrawing rats do not produce antinociception in response to acute administration of morphine, the neurologic capacity for marked antinociception remains functionally intact.

I performed intra-accumbens microinjections of opioid antagonists prior to the induction of capsaicin-induced antinociception to determine if any of the nucleus accumbens opioid receptors (μ, δ or κ) still contribute to antinociception during morphine withdrawal. These antagonist experiments demonstrated that in the withdrawing animal the μ-opioid receptor alone was required for capsaicin-induced antinociception; the δ-opioid receptor was no longer required. Thus, although noxious stimulation consistently induces potent antinociception whether the animal is naïve, tolerant or withdrawing from chronic exposure to morphine, the nucleus accumbens opioid receptor dependence of this effect varies with each state. In the naïve state both μ-and δ-opioid receptors are

necessary for capsaicin-induced antinociception while neither is involved in morphine-tolerant rats and in the withdrawing state only the μ-opioid receptor is required. These findings suggest the existence of remarkable adaptability in a neural system that allows the expression of an effect, in this case antinociception, regardless of the capacity of some of its component receptors to contribute normally.

Nucleus accumbens μ-opioid agonist-induced antinociception during morphine withdrawal

Unexpectedly, in morphine-withdrawing rats microinjection of the μ-opioid receptor agonist DAMGO alone could produce antinociception. Twice the dose of intra-accumbens DAMGO in the naïve rat did not produce antinociception (data not shown). One possible mechanism contributing to the appearance of μ–opioid agonist-induced antinociception during morphine withdrawal is μ-opioid receptor sensitization. Opioid withdrawal-induced μ-receptor mediated sensitization has previously been demonstrated at the behavioral level. Previous exposure to opiates produces sensitization to opiate-induced stimulation of motor behaviors (Kumar et al., 1971; Babbini and Davis, 1972; Kalivas and Duffy, 1987). Increased sensitivity to morphine's stimulant effects on dopamine release in nucleus accumbens also occurs during morphine withdrawal (Acquas and Di Chiara, 1992). Nucleus accumbens dopamine release mediates antinociception in the rat (Altier and Stewart, 1998; Altier and Stewart, 1999) and intra-accumbens DAMGO stimulates dopamine release (Yoshida et al., 1999). The sensitization of μ-opioid agonist stimulatory effects on dopamine release during morphine withdrawal might explain my finding that microinjection of DAMGO alone into nucleus accumbens produces marked antinociception during morphine withdrawal.

The role of nucleus accumbens κ-opioid receptors during morphine withdrawal

Similar to my observations in naïve and morphine-tolerant rats, pretreatment with a κ-opioid receptor agonist antagonized the antinociceptive effect of intra-accumbens DAMGO administration. However, unlike my observations in naïve rats, intra-accumbens κ-receptor agonism had no effect on noxious stimulus-induced antinociception. I proposed that κ-mediated antagonism of accumbens-mediated antinociception might result from antagonistic/inhibitory actions on dopamine release (Chapter 2). I, along with others, have shown that nucleus accumbens dopamine is important in antinociception (Chapter 3; Altier and Stewart, 1999) and both noxious stimulation (Chapter 3) and μ-/δ-opioid administration enhance dopamine release (Yoshida, et al., 1999); furthermore, it is known that intra-accumbens κ-agonism decreases dopamine release (Spanagel et al., 1992). Therefore, if it is true that activation of κ-opioid receptors blocks antinociception by inhibition of dopamine release, my current findings in withdrawing rats indicate that this effect on dopamine occurs in the case of DAMGO-induced, but not capsaicin-induced, antinociception. This dissociation could indicate that partially non-overlapping separate dopaminergic circuits are involved in these two antinociceptive mechanisms.

The physiological function of this intra-accumbens κ-mediated antianalgesia system is not known, however, it could play a role in the induction or facilitation of the signs and symptoms related to withdrawal from opiates. For example, dynorphin, an endogenous ligand for κ-receptors is known to increase in nucleus accumbens during opioid withdrawal (Trujillo and Akil, 1990), and κ-opioid receptor activation has been

implicated in aversion and dysphoria during withdrawal (Koob, et al., 1989b). My findings suggest that κ-opioid agonism could contribute to the antianalgesia experienced during morphine withdrawal.

In summary, I have confirmed that morphine-withdrawing rats do not exhibit an antinociceptive response to high-dose morphine; however, a noxious stimulus produces the same level of antinociception generated in the naïve rat. The δ-opioid receptor is no longer required for either pharmacologic or physiologic activation of nucleus accumbens pain control mechanisms. On the other hand, the nucleus accumbens μ-opioid receptor remains necessary for capsaicin-induced antinociception. Interestingly, the antinociceptive response induced by intraplantar capsaicin is no longer antagonized by κ-opioid agonism during the withdrawal state. However, similar to my finding with antinociception produced by the co-injection of a μ- and δ-agonist in naïve animals, the μ-opioid mediated effect is antagonized by intra-accumbens κ-opioid agonism. Persistence of κ-opioid receptor-mediated antagonism of μ-opioid analgesia could contribute to the aversion that accompanies opioid withdrawal while the enhanced μ-opioid mediated effect might shed light on the biochemical mechanism underlying the compulsive use of opiates. These pharmacologic findings lend further support to the hypothesis that pleasure-seeking and withdrawal-avoidance exist as parallel motivational factors in opiate addiction (Schulteis and Koob, 1996).

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Table 1. Statistical summary.

	ANOVAs				Tukey post hocs	
	Effects	DF	*F*	*p*	Groups	*p*
Fig. 2	Tx	1,10	16.445	0.002	n/a*	
	Time	3,30	5.722	0.024		
	Time × tx	3,30	1.573	0.239		
Fig. 3	Tx	1,20	0.752	0.396	n/a*	
	Time	3,60	0.249	0.806		
	Time × tx	3,60	0.168	0.869		
Fig. 4	Tx	3,24	3.925	0.021	1 v 2	1.000
	Time	3,72	0.793	0.464	1 v 3	0.022
	Time × tx	9,72	0.806	0.576	1 v 4	0.917
					2 v 3	0.046
					2 v 4	0.931
					3 v 4	0.202
Fig. 5	Tx	2,13	13.185	<0.001	1 v 2	0.917
	Time	3,39	7.729	0.004	1 v 3	0.001
	Time × tx	6,39	6.482	0.002	2 v 3	0.002
Fig. 6	Tx	2,16	10.283	0.001	1 v 2	0.035
	Time	3,48	4.998	0.004	1 v 3	0.001
	Time × tx	6,48	4.421	0.001	2 v 3	0.401
Fig. 7	Tx	1,20	1.134	0.300	n/a*	
	Time	3,60	0.190	0.851		
	Time × tx	3,60	0.130	0.899		

The discussion and conclusions of this study are based largely on the main effect of treatment ("Tx") and the Tukey post hoc analyses shown in the extreme right column; however, the main effect of time ("Time") and the time × treatment interaction ("Time × tx") are shown for completeness. The identity of the groups in the post hoc column is indicated by the numbers which are given in each of the respective figures.

* Post hoc analysis not done because there were only two groups.

Figure 1.

Location of injections. Filled circles are considered to be within the target area of nucleus accumbens; note that they mostly fall within the area of the core.

Figure 1

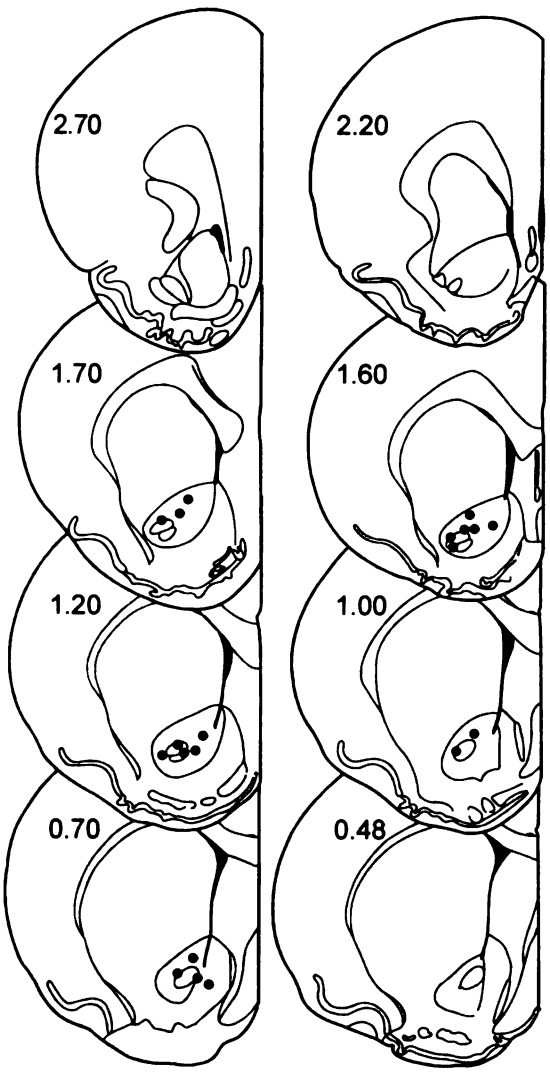


Figure 2.

Effect of subcutaneously administered morphine (10 mg/kg) in naïve and morphine-withdrawing rats. Note that this dose of morphine produced little or no antinociception in morphine-withdrawing rats. In this and subsequent figures antinociception is plotted as per cent attenuation from baseline of the JOR EMG amplitude on the Y-axis (i.e., greater antinociception is represented as higher positive numbers). Baseline JOR recordings were obtained prior to interventions. Time 0 on the X-axis represents the time at which the last (or only) treatment was given for each group. Data are plotted as mean ± s.e.m. Number of rats in each group is shown in parentheses.

Figure 2

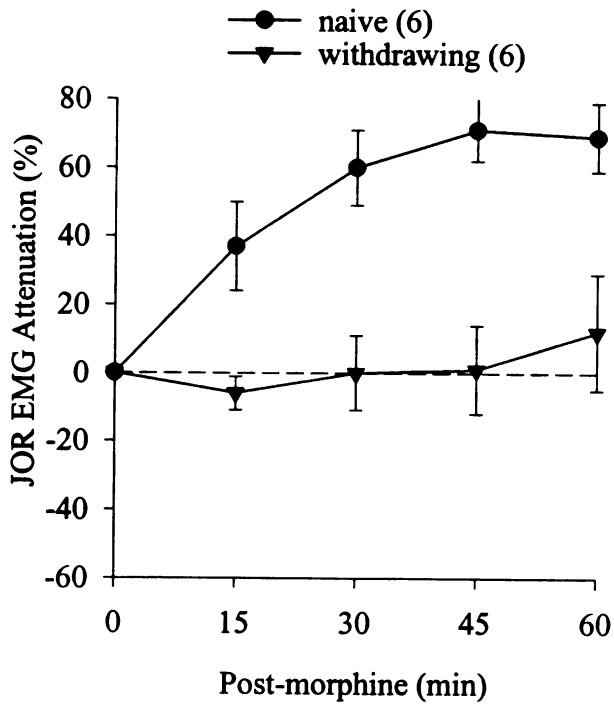

naive (6)
withdrawing (6)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
-40
-60
0
15
30
45
60
Post-morphine (min)

Figure 3.

Effect of intraplantar capsaicin in naïve and morphine-withdrawing rats. Note that morphine withdrawal did not attenuate capsaicin-induced antinociception.

Figure 3

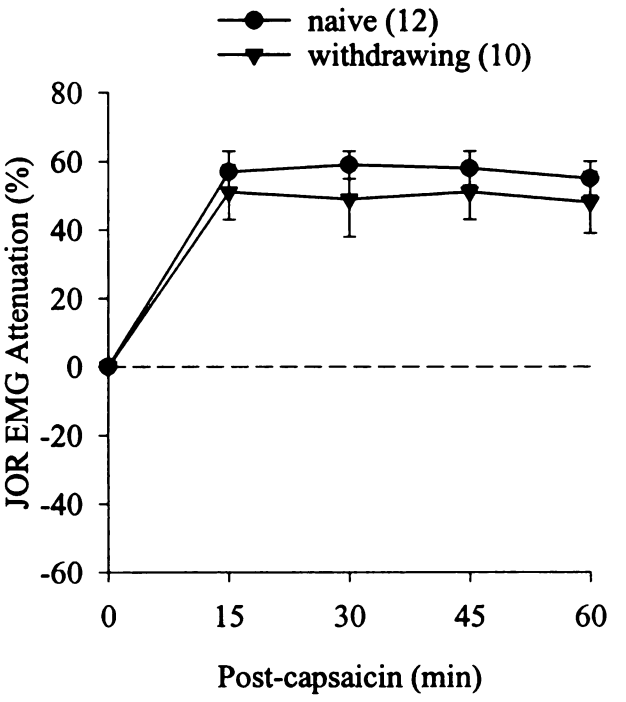

naive (12)
withdrawing (10)
80
60
40
20
0
-20
-40
-60
JOR EMG Attenuation (%)
0
15
30
45
60
Post-capsaicin (min)

Figure 4.

Effect of intra-accumbens administration of selective opioid receptor antagonists on capsaicin-induced antinociception. Note that only CTOP (μ-antagonist) significantly attenuated the antinociceptive effect of capsaicin. In this and subsequent figures group numbers, preceding group names, refer to the Tukey post hoc analyses in Table 1.

Figure 4

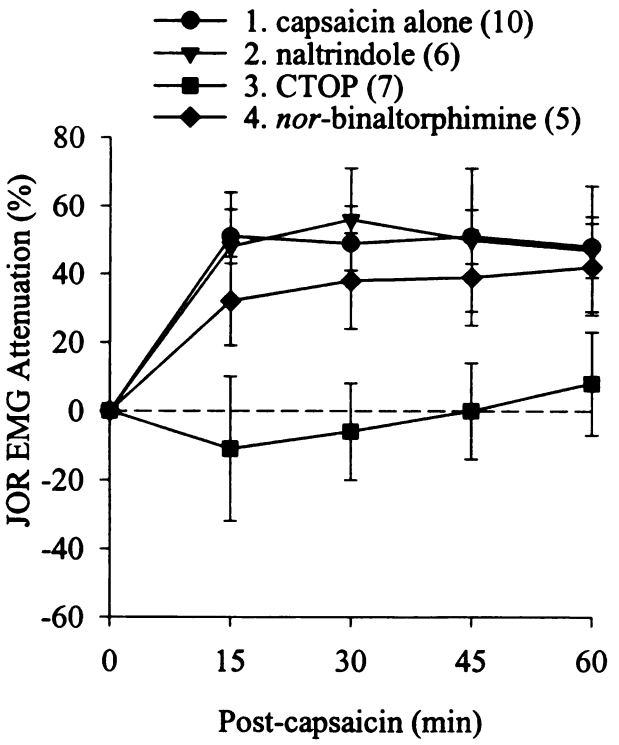

1. capsaicin alone (10)
2. naltrindole (6)
3. CTOP (7)
4. nor-binaltorphimine (5)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
-40
-60
0
15
30
45
60
Post-capsaicin (min)

Figure 5.

Effect of intra-accumbens administration of selective opioid receptor agonists. DAMGO and DPDPE were administered either alone or in combination. Two doses (150 ng and 450 ng) of each agonist were administered. Dose #1 (150 ng) was administered at the beginning of the experiment. After recording the JOR 15 and 30 minutes post-administration, dose #2 was administered; the JOR was again recorded 15 and 30 minutes later. DAMGO by itself produced significant antinociception; DPDPE alone did not affect the JOR nor did it enhance the effect of DAMGO.

Figure 5

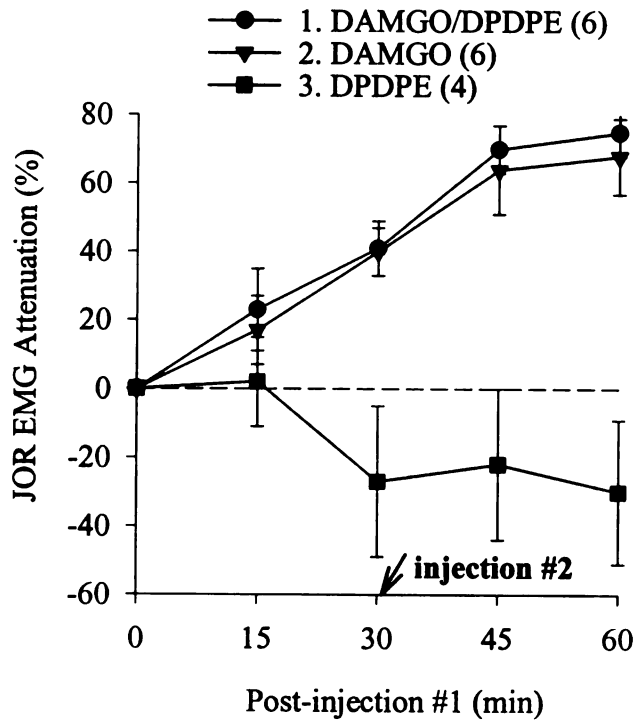

1. DAMGO/DPDPE (6)
2. DAMGO (6)
3. DPDPE (4)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
-40
-60
injection #2
0
15
30
45
60
Post-injection #1 (min)

Figure 6.

Effect of intra-accumbens administration of the κ-agonist U69,593 on the antinociception produced by the μ-agonist DAMGO. U69,593 significantly inhibited the antinociceptive effect of DAMGO.

Figure 6

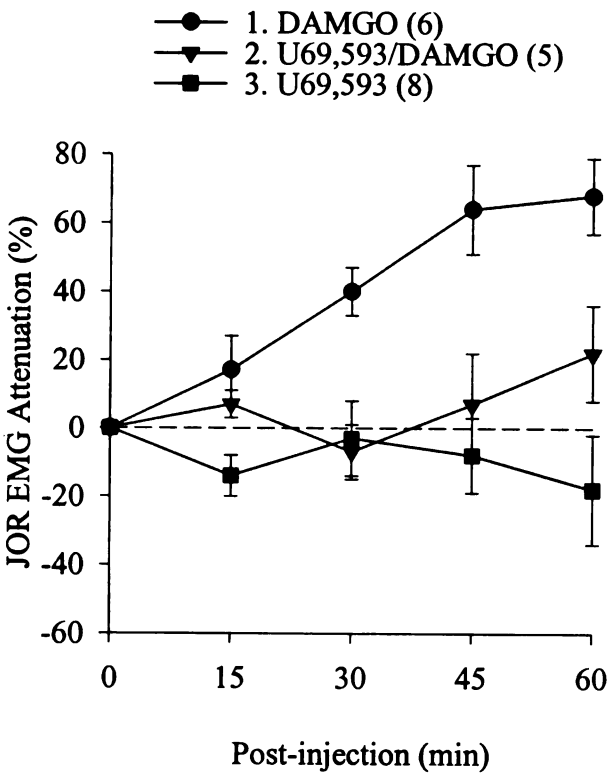
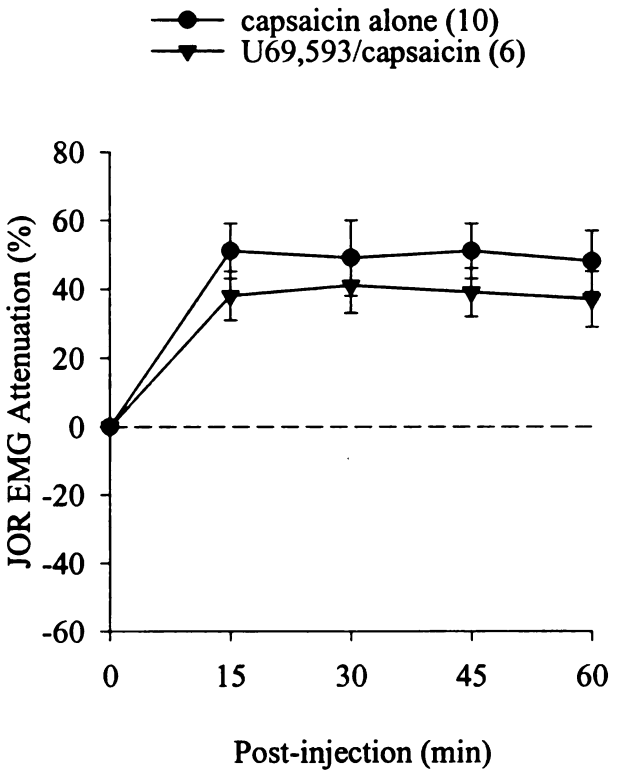

1. DAMGO (6)
2. U69,593/DAMGO (5)
3. U69,593 (8)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
-40
-60
0
15
30
45
60
Post-injection (min)

Figure 7.

Effect of intra-accumbens administration of the κ-agonist U69,593 on the antinociception produced by intraplantar capsaicin administration. U69,593 did not significantly affect capsaicin-induced antinociception.

Figure 7


capsaicin alone (10)
U69,593/capsaicin (6)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
-40
-60
0
15
30
45
60
Post-injection (min)

Chapter 5

Nicotine Withdrawal Hyperalgesia and Opioid-Mediated Analgesia Depend on Nicotine Receptors in Nucleus Accumbens

Abstract

The nucleus accumbens, as part of the mesolimbic dopaminergic reward pathway, mediates both addiction to and withdrawal from substances of abuse. In addition, activity of substances of abuse such as opioids in the nucleus accumbens has been implicated in pain modulation. Because nucleus accumbens nicotinic receptors are important in nicotine addiction and because nicotinic activity can interact with opioid action, I investigated the contribution of nucleus accumbens nicotinic receptors to opioid-mediated analgesia/antinociception. The response of the nociceptive jaw-opening reflex to opioids was studied in the rat, both before and during chronic nicotine exposure. In nicotine-naïve rats, intra-accumbens injection of the nicotinic receptor antagonist mecamylamine blocked antinociception produced by either systemic morphine, intra-accumbens co-administration of a μ- and a δ-opioid agonist, or noxious stimulation (i.e., subdermal capsaicin in the hind paw); intra-accumbens mecamylamine alone had no effect. The antinociceptive effect of either morphine or noxious stimulation was unchanged during nicotine tolerance; however, intra-accumbens mecamylamine lost its ability to block antinociception produced by either treatment. Intra-accumbens mecamylamine by itself precipitated significant hyperalgesia in nicotine tolerant rats

which could be suppressed by noxious stimulation as well as by morphine. These results indicate that nucleus accumbens nicotinic receptors play an important role in both opioid- and noxious stimulus-induced antinociception in nicotine-naïve rats. This role was attenuated in the nicotine-dependent state. The suppression of withdrawal hyperalgesia by noxious stimulation suggests that pain can ameliorate the symptoms of withdrawal, thus suggesting a possible mechanism for pain seeking behavior.

Introduction

Nicotine, through tobacco consumption, is one of the most frequently used addictive drugs worldwide, affecting more than 1.2 billion people in 1998 (Corrao et al., 2000) and resulting in extensive morbidity and mortality. Furthermore, precipitation of a withdrawal syndrome following smoking cessation poses a marked problem in the treatment of nicotine addiction (U.S. Surgeon General's Report, 1988). It has been reported that the relapse rate for individuals in smoking cessation programs is approximately 80% (Stitzer and Gross, 1988). Nicotine withdrawal is characterized by dysphoria, insomnia, irritability, anxiety, craving, cognitive deficits, and physical discomfort, within hours after the last cigarette (Shiffman and Jarvik, 1976; Hughes et al., 1991; Hughes et al., 1992; American Psychiatric Association, 1994), and pain may also be associated with nicotine withdrawal (Hughes, et al., 1992; Smith et al., 1996; Allen et al., 2000). Rats chronically treated with nicotine also exhibit signs of withdrawal either after administration of a nicotine receptor antagonist or after cessation of nicotine administration (Malin et al., 1992; Malin et al., 1994; Hildebrand et al., 1997).

It has been suggested that nucleus accumbens, as part of the mesolimbic dopaminergic pathway, mediates both the reinforcing effects of nicotine and the aversive aspects of nicotine withdrawal (Clarke, 1990; Corrigall, 1991; Hildebrand et al., 1998). In addition to this role in mediating effects of substances of abuse, nucleus accumbens plays a role in pain modulation (Dill and Costa, 1977; Yu and Han, 1990; Gear and Levine, 1995; Altier and Stewart, 1999; Gear et al., 1999). It is even possible that many of the accumbens mechanisms implicated in addiction also may be important in analgesia. For example, nucleus accumbens dopaminergic mechanisms, which are activated both by

opioids (Johnson and North, 1992) and nicotine (Kaiser and Wonnacott, 1999), have been proposed to play an important role in antinociception (Altier and Stewart, 1999).

Noxious (painful) stimulation (i.e., capsaicin injection in the hindpaw) induces profound antinociception that is mediated by both dopaminergic and opioidergic mechanisms in nucleus accumbens (Gear, et al., 1999). Since reward circuitry appears to contribute to pain modulation and since alterations in pain perception can occur during states of nicotine exposure and withdrawal, investigation of the nociceptive action of nicotinic receptors in the nucleus accumbens should provide insight into mechanisms of nicotine addiction. To investigate this role I evaluated the contribution of nucleus accumbens nicotinic receptors to antinociception produced by systemic morphine, intra-accumbens opioid agonists and noxious stimulation. In addition, I evaluated whether the role of nucleus accumbens nicotinic receptors to antinociception produced in these settings is altered during nicotine tolerance and withdrawal.

Materials and Methods

As in previous studies (Gear and Levine, 1995; Gear, et al., 1999), the nociceptive jaw-opening reflex (JOR) was used to measure changes in nociceptive responses. Experiments were performed on 280 - 380 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA). These animals were maintained in the University of California, San Francisco, animal care facility, in accordance with applicable university policies. The UCSF Committee on Animal Research approved experimental protocols.

Anesthesia

Animals were anesthetized by intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma, St. Louis, MO). This anesthetic protocol was chosen for anesthesia because it provides a stable jaw-opening reflex electromyographic (EMG) signal over the time period required to complete the experiments (Gear and Levine, 1995; Gear, et al., 1999).

Electrode implantation

To evoke the JOR, a bipolar stimulating electrode, fabricated from two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, Inc, South San Francisco, CA). A bipolar recording electrode, consisting of two insulated copper wires (36 AWG) with 4 mm of insulation removed, was inserted into the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

Jaw-opening reflex

At the beginning of each experiment stimulation current was set at 3 times the threshold current for detecting the JOR electromyogram (EMG). Changes in nociception were measured as changes in JOR electromyographic signal amplitude (Gear and Levine, 1995; Gear, et al., 1999). Each data point consisted of the average peak-to-peak amplitude of 12 consecutive JOR EMG signals evoked by stimulating the tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Baseline amplitude was defined as the average of the last 3 data points, recorded at 5 minute intervals, before an experimental intervention. Effects of experimental interventions are expressed as the mean percentage change ± s.e.m. from the baseline for each experimental group.

Cannula placement

Stainless steel guide cannulae (23 gauge) were stereotactically positioned and cemented with orthodontic resin (L.D. Caulk Co., Milford, DE) to allow injections via insertion of a 30 gauge stainless steel injection cannula, which extended beyond the guide cannulae 2 mm, connected to a 2 μl syringe (Hamilton, Reno, NV). Supraspinal injections were bilateral with volumes of 0.5 μl in all experiments. These injections were carried out over a period of 90 seconds, and the injection cannulae were left in place an additional 30 seconds after injection. The stereotaxic instrument was set to the following coordinates for nucleus accumbens (from bregma) 1.2 mm rostral, 7.2 mm ventral, and ± 1.8 mm lateral from the midline. These coordinates place injections into the core area of nucleus accumbens. Injection sites were verified by histological examination (100 μm sections stained with cresyl violet acetate) and were plotted on coronal sections adapted

from the atlas of Paxinos and Watson (Paxinos and Watson, 1986) and are shown in Fig. 1.

Drugs

Capsaicin (Sigma, St. Louis, MO) was dissolved in Tween 80 (5%), ethanol (5%) and saline (90%) to a concentration of 5 μg/μl. Subdermal capsaicin injection volume was 50 μl (i.e., 250 μg capsaicin) in all experiments. Mecamylamine, [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and D-Pen^{2,5}-enkephalin (DPDPE) (all from Sigma) were dissolved in phosphate buffered saline (PBS).

Nicotine tolerance

Nicotine tolerance was induced by infusion of nicotine tartrate (Sigma), dissolved in de-ionized water and adjusted to pH 7.2 - 7.4, at the rate of 9 mg/kg body weight per day with subcutaneously implanted osmotic minipumps (Alzet, #1007D, Newark, DE) for 7 days. This dose of the tartrate salt (equivalent to 3.16 mg/kg/day of nicotine base) induced substantial withdrawal effects in rats (Epping-Jordan et al., 1998) and was reported to produce plasma nicotine levels of 44 ng/ml, comparable to those reported for smokers consuming 30 cigarettes daily (Benowitz, 1988).

Data analysis

A two-way repeated measures ANOVA with one between subjects factor (i.e., treatment) and one within subjects factor (i.e, time) was used to determine if there were significant ($p \leq 0.05$) differences in antinociceptive responses among the groups. For each ANOVA the Mauchley criterion was used to determine if the assumption of sphericity for the within-subjects effects was met; if the Mauchley criterion was not satisfied, Greenhouse-Geisser adjusted *p* values are presented. If there was a significant

between-subjects main effect of treatment group, the Tukey test was employed post hoc to determine the basis of the significant difference. The alpha level for post hoc contrasts employed to identify the time points at which there was a significant difference from baseline within a particular group was adjusted using a Bonferroni-type correction (e.g., $p = 0.05 \div 4 = 0.0125$ for 4 time points compared to baseline).

Results

Nucleus accumbens nicotinic receptors in opioid-mediated antinociception

The role of nucleus accumbens nicotinic receptors in opioid-mediated antinociception was studied by injecting the nicotinic receptor antagonist mecamylamine (0.6 μg) into nucleus accumbens 10 minutes prior to subcutaneous morphine (5 mg/kg) administration (Figure 2*a*). Mecamylamine completely blocked the antinociceptive effect of systemic morphine (Table 1), indicating that nucleus accumbens nicotinic receptors play an important role in systemic opioid-mediated antinociception. The ability of mecamylamine to antagonize analgesia was decreased in animals administered a higher dose of morphine (10 mg/kg, Fig. 2*b*). Although mecamylamine tended to reduce the effect of morphine at the later time points, this effect was not significant (Table 1), indicating that the role of intra-accumbens nicotinic receptors in the antinociceptive effect morphine is diminished with higher doses. To determine if nucleus accumbens was the site of action of mecamylamine, extra-accumbens (i.e., offsite) injections were performed. Attenuation of morphine antinociception by mecamylamine was significantly greater with intra-accumbens injections compared to extra-accumbens injections (Table 1, Fig. 1). Intra-accumbens mecamylamine administered alone did not affect the JOR. In another study (Chapter 2) I observed that while intra-accumbens infusion of a μ- or δ-opioid agonist alone does not produce antinociception, a combination of the two does. Intra-accumbens morphine also induces antinociception (Dill and Costa, 1977; Yu and Han, 1990), perhaps due to its combined μ- and δ-opioid agonist actions. To determine whether nicotinic receptors are downstream from opioid receptors in a nucleus accumbens antinociceptive circuit, I tested if antinociception induced by opioids in

nucleus accumbens is reduced by intra-accumbens mecamylamine administration. A combination of the selective μ-opioid receptor agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO, 150 ng) and the selective δ-opioid receptor agonist D-Pen^{2,5}-enkephalin (DPDPE, 150 ng), that produces antinociception when co-administered at this dose (Schmidt et al., submitted), was administered into nucleus accumbens either alone or with mecamylamine (0.6 μg, Figure 2*c*). The opioid combination produced antinociception and mecamylamine blocked the antinociceptive effect produced by the intra-accumbens combination (Table 1).

Opioid-mediated antinociception during nicotine tolerance

Since nicotinic receptors in nucleus accumbens contribute to systemic morphine antinociception, I determined if chronic exposure to nicotine induces tolerance to the antinociceptive effects of morphine. Therefore, the antinociceptive effect of systemic morphine (5 mg/kg, s.c.) in nicotine-tolerant and nicotine-naïve rats was compared (Fig. 3). The effect of morphine in these two groups was not significantly different (Table 1), indicating that nicotine tolerance does not result in morphine tolerance.

To determine if the role played by nicotinic receptors in morphine-induced antinociception changes during nicotine tolerance, intra-accumbens mecamylamine (0.6 μg) was administered either alone or 10 minutes prior to subcutaneous morphine (5 mg/kg, Fig. 4*a*) in nicotine tolerant rats (see Methods). Because these rats were tolerant to nicotine, intra-accumbens mecamylamine would be expected to precipitate withdrawal. Intra-accumbens mecamylamine induced a significant (p=0.004) enhancement of the jaw-opening reflex (Table 1) suggesting the induction of withdrawal hyperalgesia, which, however, was prevented by morphine administration. Also, although in nicotine-naïve

rats mecamylamine completely blocked the antinociceptive effect of the same dose of morphine (Fig. 2*a*), in nicotine-tolerant rats there was no significant difference between the groups receiving morphine with or without mecamylamine (Table 1, p= 0.437), suggesting that in the nicotine-tolerant rat morphine antinociception is intact but with a diminished role for intra-accumbens nicotinic receptors. To determine if intra-accumbens nicotinic receptors still contribute to morphine antinociception, even though such contribution is diminished, the ability of intra-accumbens mecamylamine to inhibit the antinociceptive effect of a smaller dose of morphine (2.5 mg/kg) in nicotine-tolerant rats was tested. Mecamylamine (0.6 μg) was able to block the antinociceptive effect of this smaller dose of morphine (Fig. 4*b*, Table 1). Taken together, these findings indicate that nicotine tolerance reduces but does not eliminate the role of intra-accumbens nicotinic receptors in morphine antinociception, and that mecamylamine-precipitated withdrawal in rats chronically treated with nicotine results in a marked hyperalgesia that is antagonized by morphine administration.

Nucleus accumbens nicotinic receptors in noxious stimulus-induced antinociception

Noxious stimulation, in the form of intra-plantar capsaicin administration, induces antinociception that is mediated by opioid and dopamine receptors in the nucleus accumbens (Gear, et al., 1999). In the current study, I examined the role of the nucleus accumbens nicotinic receptors in capsaicin-induced antinociception. Intra-accumbens mecamylamine (0.6 μg) completely blocked the antinociceptive effect of intra-plantar capsaicin administered 10 minutes later (Fig. 5, Table 1), indicating that noxious stimulus-induced antinociception is mediated by intra-accumbens nicotine receptors similarly to opioid-induced antinociception. The effect of extra-accumbens

mecamylamine (i.e., offsite) administration was significantly less than that of intra-accumbens mecamylamine (data not shown), supporting the suggestion that nucleus accumbens is the site of action (Table 1, injection sites shown in Fig. 1).

I compared the antinociceptive effect of intraplantar capsaicin in nicotine-tolerant and nicotine-naïve rats (Fig. 6). Similar to the result with systemic morphine, there was no significant difference in these groups (Table 1) indicating that nicotine-tolerance does not induce tolerance to noxious stimulus-mediated antinociception.

To determine if noxious stimulation, like systemic morphine, could mask mecamylamine-induced hyperalgesia in nicotine tolerant rats, the nicotinic antagonist mecamylamine (0.6 μg) was administered into nucleus accumbens 10 minutes prior to intraplantar capsaicin administration (Fig. 7). Mecamylamine-precipitated withdrawal hyperalgesia was abolished by capsaicin, and mecamylamine did not significantly affect capsaicin-induced antinociception (Table 1), indicating that intra-accumbens nicotinic receptors do not play a significant role in the antinociceptive effect of capsaicin, but that noxious (painful) stimuli can *inhibit* the hyperalgesic effect of precipitated nicotine withdrawal (Table 1).

Discussion

Continued nicotine intake, through smoking, produces tolerance and physiological dependence, which severely impedes attempts at smoking cessation. Abstinence is often short-lived, relapse rates are high and most people resume smoking (Stitzer and Gross, 1988; Hughes, et al., 1992). Because achiness and pain-like symptoms can be a component of nicotine withdrawal and hamper attempts at smoking cessation (Hughes, et al., 1992; Smith, et al., 1996; Allen, et al., 2000), I tested if chronic exposure to nicotine affects the contribution of the nicotinic receptor to pain modulation and particularly to opioid-mediated antinociception.

A striking finding was that agonism in nucleus accumbens nicotinic receptors has an important role in systemic morphine antinociception, although this role is decreased at very high doses of morphine. Although systemically administered morphine activates opioid receptors throughout the neuraxis (Yaksh et al., 1976; Yaksh and Rudy, 1977; Yeung et al., 1977; Yaksh, 1981; Yaksh et al., 1988; Tseng and Wang, 1992), this site-specific intervention produced a large inhibitory effect on the antinociceptive effect of a moderately high dose of systemically administered morphine. Given that morphine's action at different central nervous system sites produces a greater-than-additive ("multiplicative") antinociceptive effect (Yeung and Rudy, 1980), it is not surprising that blocking its action at one site can result in a marked inhibition of systemic opioid-induced antinociception. Consistent with this antagonism of systemic morphine antinociception, intra-accumbens mecamylamine also blocked the antinociceptive effect of intra-accumbens opioid administration suggesting that the nicotinic receptors in the nucleus accumbens are facilitative at the same level or are downstream from accumbens

opioid receptors. Nucleus accumbens nicotinic receptor antagonism also blocked noxious stimulus-induced antinociception, compatible with a previous study implicating nucleus accumbens mechanisms in this form of antinociception (Gear, et al., 1999).

Current evidence suggests that nicotine and morphine produce similar effects within nucleus accumbens. Nicotine and morphine both increase dopamine transmission in nucleus accumbens; this increase in dopamine release can be blocked by intra-ventral tegmental area (VTA) injections of mecamylamine and naloxone, respectively (Nisell et al., 1994; Pontieri et al., 1996; Tanda and Di Chiara, 1998). Microinjection of nicotine and morphine into the VTA lead to dopamine release in nucleus accumbens (Panagis et al., 1996). Similar to my current finding that intra-nucleus accumbens mecamylamine antagonizes the antinociceptive effect of systemic morphine, others have shown that an intra-VTA injection of naloxone blocks the dopamine release produced by systemic nicotine (Tanda and Di Chiara, 1998). These similarities between the actions of nicotine and morphine in nucleus accumbens suggests that the two substances of abuse might involve overlapping mechanisms, which may explain modulation of morphine antinociception by mecamylamine.

I also found that a state of nicotine tolerance did not significantly reduce capsaicin- or morphine-induced antinociception, and that mecamylamine no longer blocked capsaicin-induced antinociception and was able to block the antinociceptive effect of morphine but only at a lower morphine dose. It is unclear how the changing role of nucleus accumbens nicotinic receptors in antinociception during nicotine tolerance occurs while leaving both morphine- and capsaicin-induced antinociception unchanged. However, intra-nucleus accumbens mecamylamine itself induced a marked hyperalgesic

effect as indicated by significant enhancement of the JOR indicating that nicotinic receptors maintain a role in nociceptive modulation, even during nicotine tolerance. Since this large withdrawal hyperalgesia is blocked by administering systemic opioids or activating endogenous opioids (e.g., by capsaicin), there appears to be a potent nicotine-opioid interaction in the nucleus accumbens present in the tolerant state. The hyperalgesic effect of nicotine withdrawal might contribute to tobacco addiction and difficulty in attempts at cessation.

In summary, I demonstrate that nucleus accumbens nicotinic receptors play a role in pain modulation in both naïve and nicotine-dependent animals. This suggestion is supported by my findings that nucleus accumbens nicotinic receptor antagonism by mecamylamine blocked systemic morphine antinociception in naïve animals and precipitated withdrawal hyperalgesia in nicotine-tolerant animals. Nicotine withdrawal hyperalgesia has also been reported after systemic mecamylamine administration (Yang et al., 1992). The marked nicotinic withdrawal hyperalgesia was blocked by systemically administered morphine or by noxious stimulation, indicating that a painful stimulus can itself block the hyperalgesia produced by withdrawal from substances of abuse. Since the protocol for chronic nicotine administration used in this study results in plasma levels approximately the same as those produced by smoking in humans, these findings may help to explain at least part of the discomfort associated with smoking cessation. The finding that nicotine withdrawal hyperalgesia is blocked by noxious stimulation may be relevant to clinical syndromes characterized by an addictive form of self-injurious behavior (Casner et al., 1996; Roth et al., 1996).

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Table 1. Statistical summary.

	Effects	DF	*F*	*p*	Tukey post hocs Groups	*p*
Fig. 2*a*	Tx	1,24	4.085	0.030	1 v 2	0.050
	Time	3,72	5.635	0.002	1 v 3	0.049
	Time × tx	6,72	1.867	0.098	2 v 3	0.949
Fig. 2 offsite *(not plotted)*	Tx	1,17	5.279	0.035		
	Time	3,51	2.007	0.150	n/a[a,b]	
	Time × tx	3,51	1.040	0.364		
Fig. 2*b*	Tx	2,14	8.028	0.005	1 v 2	0.990
	Time	3,42	10.992	<0.001	1 v 3	0.007
	Time × tx	6,42	2.833	0.047	2 v 3	0.021
Fig. 2*c*	Tx	2,22	10.335	0.001	1 v 2	0.001
	Time	3,66	1.476	0.240	1 v 3	0.006
	Time × tx	6,66	3.134	0.025	2 v 3	0.861
Fig. 3	Tx	1,12	1.620	0.227		
	Time	3,36	5.818	0.005	n/a[b]	
	Time × tx	3,36	0.437	0.689		
Fig. 4a	Tx	2,18	14.805	<0.001	1 v 2	0.435
	Time	3,54	0.103	0.894	1 v 3	<0.001
	Time × tx	6,54	1.358	0.269	2 v 3	0.004
Fig. 4b	Tx	2,23	15.819	<0.001	1 v 2	0.025
	Time	3,69	0.403	0.664	1 v 3	<0.001
	Time × tx	6,69	1.947	0.121	2 v 3	0.038
Fig. 4, mec alone	Time	4,36	3.995	0.029	BL v 15	0.105
					BL v 30	0.027
					BL v 45	0.033
					BL v 60	0.004
Fig. 5	Tx	2,22	22.311	<0.001	1 v 2	<0.001
	Time	3,66	1.265	0.291	1 v 3	<0.001
	Time × tx	6,66	2.727	0.044	2 v 3	0.999
Fig. 5 offsite *(not plotted)*	Tx	1,15	5.855	0.029		
	Time	3,45	9.643	0.001	n/a[b,c]	
	Time × tx	3,45	0.660	0.519		
Fig. 6	Tx	1,18	1.355	0.260		
	Time	3,54	2.074	0.150	n/a[b]	
	Time × tx	3,54	1.092	0.338		
Fig. 7	Tx	2,22	24.658	<0.001	1 v 2	0.982
	Time	3,66	0.752	0.480	1 v 3	<0.001
	Time × tx	6,66	1.358	0.263	2 v 3	<0.001

The discussion and conclusions of this study are based largely on the main effect of treatment ("Tx") and the Tukey post hoc analyses shown in the extreme right column. The main effect of time ("Time") and the time × treatment interaction ("Time × tx") are shown for completeness. The identity of the groups in the post hoc column is indicated by the numbers, which are given in each of the respective figures. In the case of "Fig. 4,

mec alone" the effect of time was significant; therefore, post hoc orthogonal contrasts comparing the mean for each time point to baseline ("BL") are shown.

[a] The results of the two-way ANOVA comparing the effect of systemic morphine (5 mg/kg) in rats that had received mecamylamine injected either into NAc (n = 11) or into sites outside of NAc (n = 8). The data for the onsite group are plotted in Fig 2a ("2"); the data for the offsite group are not plotted. All injection locations are shown in Fig. 1.

[b] Post hoc analysis was not needed because there were only two groups.

[c] The results of the two-way ANOVA comparing the effect of intraplantar capsaicin administration in rats that had received mecamylamine injected either into NAc (n = 6) or into sites outside of NAc (n = 11). The data for the onsite group are plotted in Fig 5a ("2"); the data for the offsite group are not plotted. All injection locations are shown in Fig. 1.

Figure 1.

Sites of intra-accumbens injections (filled circles) and offsite injections (open circles). All injections are plotted, but some overlap each other. Note that the injection sites are located primarily in the core of nucleus accumbens. Numbers refer to the distance of the section in mm rostral to bregma (Paxinos and Watson, 1986).

Figure 1

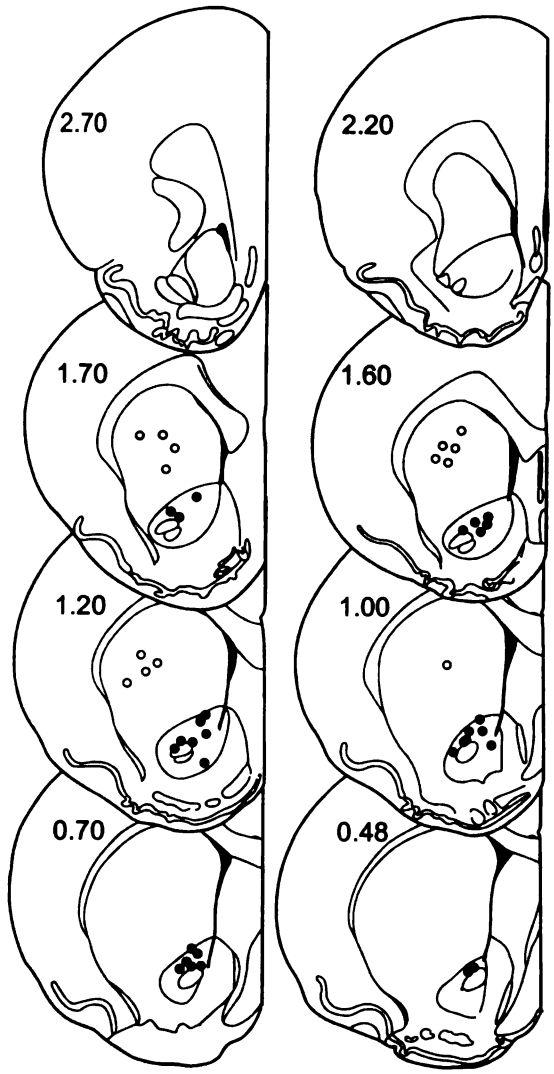


Figure 2.

Effect of intra-accumbens mecamylamine on opioid-induced antinociception in nicotine-naïve rats. *a*. Systemically administered morphine (5 mg/kg). Mecamylamine administered into nucleus accumbens blocked the effect of morphine but had no effect when injected alone. *b*. Systemically administered morphine (10 mg/kg). Mecamylamine administered into nucleus accumbens did not significantly block the effect of morphine. *c*. Intra-accumbens DAMGO/DPDPE-induced antinociception. Mecamylamine blocked the antinociceptive effect of intra-accumbens DAMGO/DPDPE administration. In this and subsequent figures antinociception is plotted as per cent attenuation from baseline of the JOR EMG amplitude on the Y-axis (i.e., greater antinociception is represented as higher positive numbers). Baseline JOR recordings were obtained prior to interventions. Time 0 on the X-axis represents the time at which the last (or only) treatment was given for each group. Data are plotted as mean ± s.e.m. Group numbers, preceding group names, refer to the Tukey post hoc analyses in Table 1. Number of rats in each group is shown in parentheses.

Figure 2

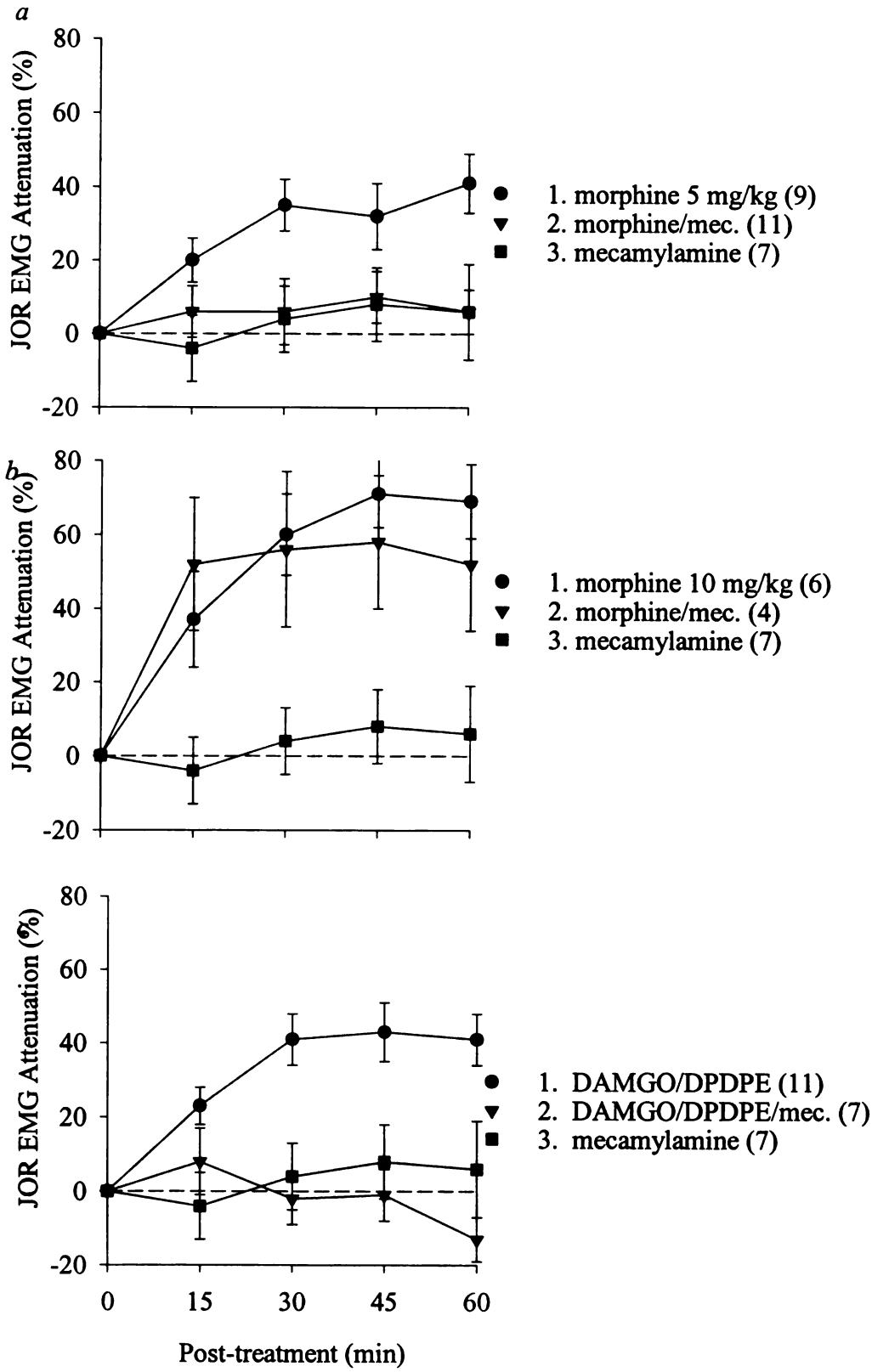

a
JOR EMG Attenuation (%)
80
60
40
20
0
-20
1. morphine 5 mg/kg (9)
2. morphine/mec. (11)
3. mecamylamine (7)
b
JOR EMG Attenuation (%)
80
60
40
20
0
-20
1. morphine 10 mg/kg (6)
2. morphine/mec. (4)
3. mecamylamine (7)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
0
15
30
45
60
Post-treatment (min)
1. DAMGO/DPDPE (11)
2. DAMGO/DPDPE/mec. (7)
3. mecamylamine (7)

Figure 3.

Effect of nicotine dependence on morphine-induced antinociception. Morphine was administered to either nicotine-naïve or nicotine-dependent rats. There was no significant difference between the two groups.

Figure 3

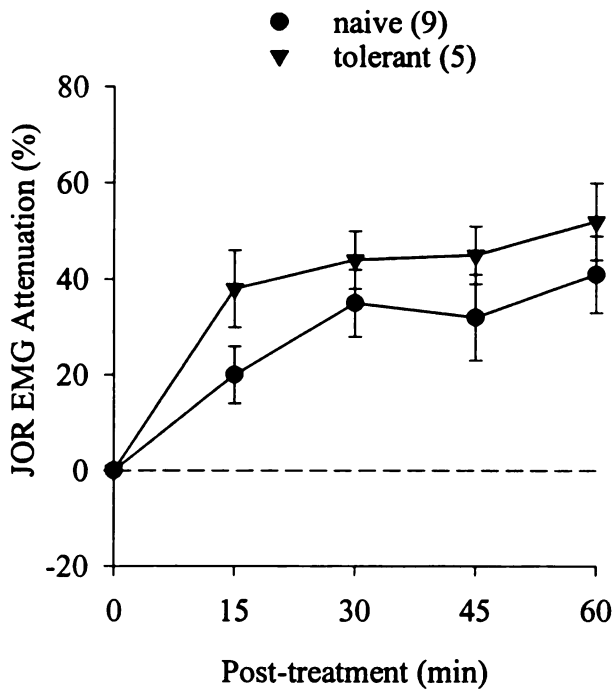

naive (9)
tolerant (5)
80
60
40
20
0
-20
JOR EMG Attenuation (%)
0
15
30
45
60
Post-treatment (min)

Figure 4. Effect of intra-accumbens mecamylamine on morphine-induced antinociception in nicotine-tolerant rats. *a.* Morphine 5 mg/kg. Mecamylamine failed to significantly attenuate the antinociceptive effect of morphine (5 mg/kg), but produced significant precipitated nicotine-withdrawal hyperalgesia shown as greater negative scores (i.e., enhancement of the JOR). This hyperalgesia did not occur in the group that received morphine (same dose) 10 min after mecamylamine administration, indicating that morphine was able to reverse the hyperalgesic effect of mecamylamine. *b.* Morphine 2.5 mg/kg. Mecamylamine completely blocked the antinociceptive effect of this lower dose of morphine.

Figure 4

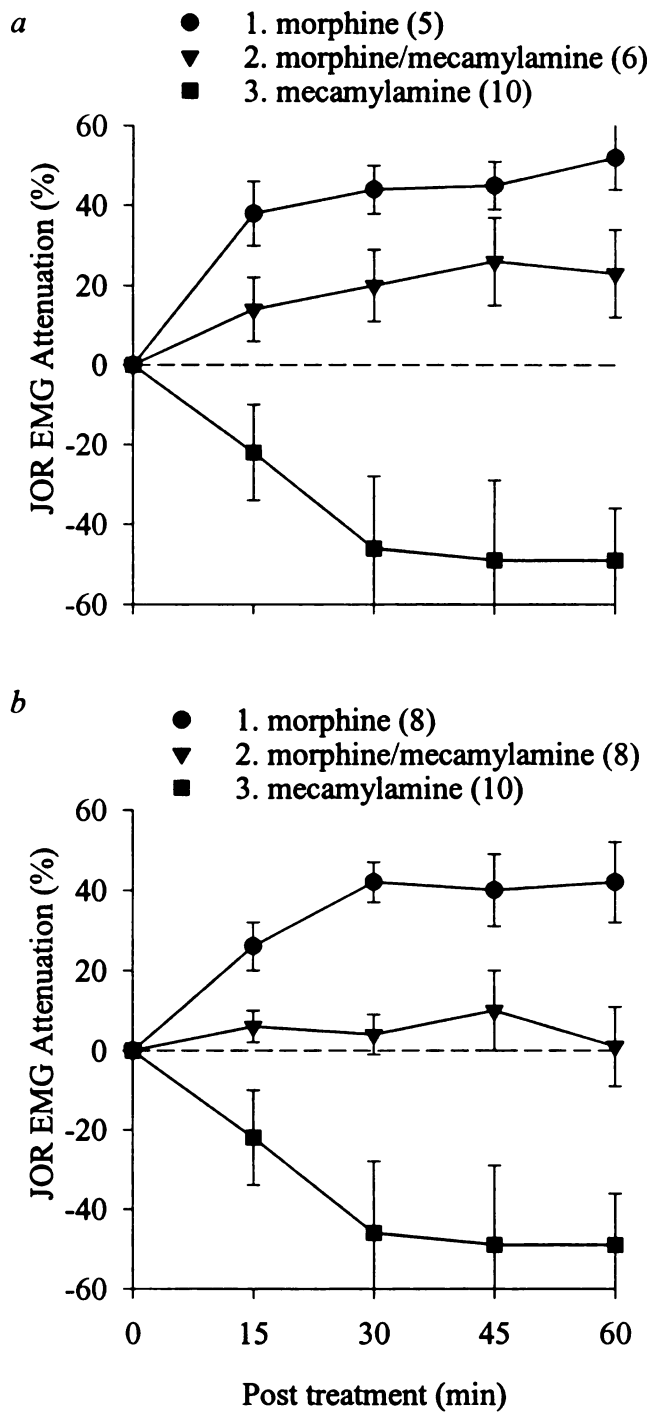

a
1. morphine (5)
2. morphine/mecamylamine (6)
3. mecamylamine (10)
JOR EMG Attenuation (%)
60
40
20
0
-20
-40
-60
b
1. morphine (8)
2. morphine/mecamylamine (8)
3. mecamylamine (10)
JOR EMG Attenuation (%)
60
40
20
0
-20
-40
-60
0
15
30
45
60
Post treatment (min)

Figure 5. Effect of intra-accumbens mecamylamine on capsaicin-induced antinociception in nicotine-naïve rats. Mecamylamine administered into nucleus accumbens blocked the capsaicin-induced antinociception. The group receiving mecamylamine alone is replotted from Fig. 2.

Figure 5

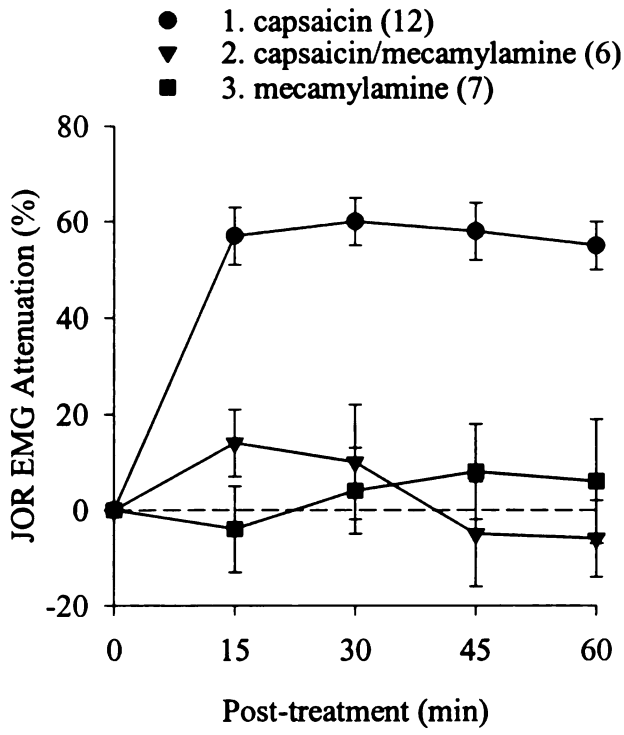

1. capsaicin (12)
2. capsaicin/mecamylamine (6)
3. mecamylamine (7)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
0
15
30
45
60
Post-treatment (min)

Figure 6. Effect of nicotine tolerance on capsaicin-induced antinociception. Capsaicin was administered to either nicotine-naïve or nicotine-dependent rats. There was no significant difference between the two groups.

Figure 6

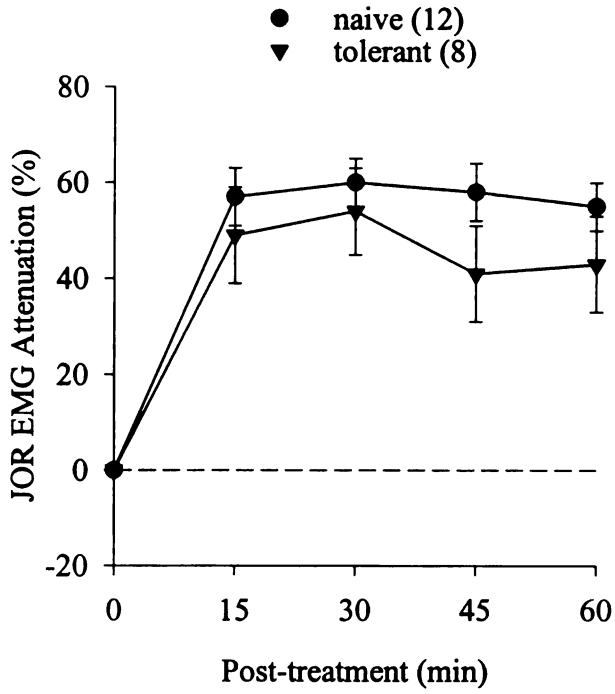
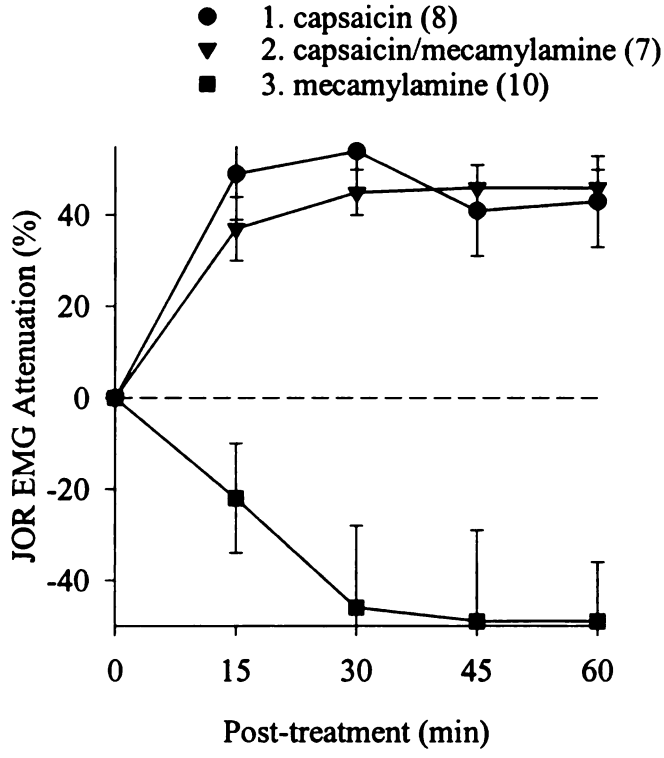

naive (12)
tolerant (8)
JOR EMG Attenuation (%)
80
60
40
20
0
-20
0
15
30
45
60
Post-treatment (min)

Figure 7. Effect of intra-accumbens mecamylamine on capsaicin-induced antinociception in nicotine-tolerant rats. Mecamylamine failed to block the effect of capsaicin, and the hyperalgesic effect of mecamylamine did not occur in the group that received capsaicin subsequent to mecamylamine administration, indicating that noxious stimulation was able to reverse the hyperalgesic effect of mecamylamine.

Figure 7


1. capsaicin (8)
2. capsaicin/mecamylamine (7)
3. mecamylamine (10)
JOR EMG Attenuation (%)
40
20
0
-20
-40
0
15
30
45
60
Post-treatment (min)

Chapter 6

Conclusion

The results of this thesis add support to the existing data implicating nucleus accumbens in pain modulation. This antinociceptive role has been clearly documented in the naïve animal; however, now we have evidence that nucleus accumbens can generate antinociception following the development of tolerance and withdrawal. Taken together, these results point to the adaptive nature of nucleus accumbens circuitry, and the conclusions can be applied to our current understanding of supraspinal mechanisms maintaining antinociception during withdrawal and tolerance, as well as, the mechanisms contributing to addiction.

In the previous chapters I have discussed the results in the context of antinociception during tolerance and withdrawal; however, the results have clear implications with opiate addiction. The current theories of opiate addiction have not considered antinociception as part of the motivation driving continued drug use. Despite this, my results dealing with antinociception during tolerance and withdrawal fit well within the principles of the opponent-process theory. This model states that the pleasant or aversive affective states produced by the drug are automatically opposed by centrally mediated mechanisms that balance the intensity of the affective state (Solomon, 1977). The state of opioid withdrawal is thought to involve a perturbation of the balance of opioid effects (Trujillo and Akil, 1991). For example, down-regulation of endogenous nucleus accumbens μ-opioid ligands and up-regulation of endogenous κ-opioid ligands are observed during withdrawal (Trujillo and Akil, 1990). This withdrawal-induced

imbalance in nucleus accumbens is hypothesized to precipitate κ-mediated dysphoria (Trujillo and Akil, 1991). Heroin addicts allowed to self-inject demonstrate tolerance to the euphoric effects within 1 to 2 weeks of administration of an escalating dose of heroin (Meyer and Mirin, 1979). The subjects report that chronic self-administration is accompanied by marked dysphoria and pronounced depression with very brief periods of extreme euphoria immediately after injecting heroin. The molecular findings of decreasing μ-opioid endogenous ligands and increasing κ-opioid endogenous ligands combined with possible μ-receptor sensitization and κ-receptor mediated anti-analgesia might define the antinociceptive component of addiction that forms the motivational process behind compulsive drug use.

Collectively, the findings from this project point to the neuroadaptivity of nucleus accumbens. Table 1 presents a summary of the findings. Interestingly, pain-induced antinociception requires opioidergic, nicotinic and dopaminergic receptors in nucleus accumbens and is intact despite morphine and nicotine tolerance. Morphine tolerance abrogates the requirement for accumbens opioid receptors but does not affect the requirement for nicotinic and dopaminergic receptors, while nicotine tolerance negates the need for nucleus accumbens nicotinic receptors. The available data at this time is insufficient to completely characterize the nucleus accumbens circuitry mediating antinociception. However, based on the combination of immunocytochemical, pharmacologic and intracellular recording studies Figure 1 presents one possible circuit mediating capsaicin-induced antinociception.

The neurons of nucleus accumbens consist of approximately 90% medium spiny neurons, which are GABAergic output neurons (Chang and Kitai, 1985), and 10%

cholinergic interneurons (Kawaguchi et al., 1995). Nucleus accumbens neurons display one of three activity states: 1) silent, 2) spontaneously firing at low, constant rates, or 3) those with a bistable membrane potential (O'Donnell and Grace, 1995). The output of nucleus accumbens appears to be pronociceptive based on our finding that intra-accumbens lidocaine is antinociceptive (unpublished data). Therefore, activation of dopaminergic and glutamatergic inputs to nucleus accumbens might inhibit medium spiny neuron output and produce antinociception(McGinty, 1999).

Nucleus accumbens receives glutamatergic input from the neocortex and dopaminergic input from the VTA (McGinty, 1999). Based on the proposed circuit nociceptive stimulation (intraplantar capsaicin) would be expected to increase nucleus accumbens dopamine release directly from the VTA and indirectly through the presynaptic cholinergic mechanism. The circuitry that activates the VTA and neocortex following intraplantar capsaicin is not known. The effect of dopamine on nucleus accumbens medium spiny neuron activity has been debated; however, coactivation of D1 and D2 receptors reduces membrane excitability (O'Donnell and Grace, 1996). Perfusion of nicotine into nucleus accumbens induces dopamine release suggesting that nicotinic receptors presynaptically regulate dopamine release (Marshall et al., 1997). Increasing evidence suggests that the behavioral effects of nicotine, including antinociception, are also mediated by endogenous opioids (Zarrindast et al., 1997; George et al., 2000). Systemic mecamylamine produces nicotine withdrawal and a reduction in nucleus accumbens dopamine release (Carboni et al., 2000). Naloxone also precipitates nicotine withdrawal, however, without a reduction in nucleus accumbens dopamine release

(Carboni, et al., 2000). Therefore, the intra-accumbens nicotinic mechanism might involve independent presynaptic regulation of both dopamine and opioids.

Kappa-opioid receptors are also located presynaptically on dopaminergic terminals and inhibit dopamine release (Spanagel et al., 1992). I have hypothesized that the antianalgesic effect of κ-opioid receptor activation with U69,593 occurs through the inhibition of dopamine release (Chapter 2). Mu- and δ-opioid receptors have been immunolocalized to GABAergic medium spiny neurons in nucleus accumbens and are thought to presynaptically inhibit medium spiny neuron activity and GABA release in the striatum (Svingos et al., 1997; Svingos et al., 1998). Based on this proposed circuit the capsaicin-induced inhibition of nucleus accumbens output would be mediated by opioid and dopamine release which is modulated by a presynaptic cholinergic mechanism. Consistent with this mechanism is the finding that systemic nicotine has been shown to inhibit the action potentials of nucleus accumbens neurons (Hakan et al., 1993). This model accounts for the dependency of capsaicin-induced antinociception on opioidergic, nicotinic and dopaminergic receptors. In addition, the redundancy in the system (inhibition of nucleus accumbens output by two separate circuits which are both modulated by acetylcholine acting on nicotinic receptors) might allow for the expression of nicotine- and dopamine-dependent antinociception during the morphine tolerant state, as well as continued antinociception during the nicotine tolerant state.

In conclusion, this thesis reports on the contribution of nucleus accumbens to antinociception in the naïve, tolerant and withdrawing rat. While the conclusions drawn from the experiments begin to provide a context for understanding the antinociceptive role of nucleus accumbens receptors, significant mechanistic issues remain unanswered.

The resistance of the nucleus accumbens μ-opioid receptor to tolerance induced by morphine (a μ-opioid agonist) has been described by others (Noble and Cox, 1996) but the underlying mechanism is not known. The possible cooperativity that exists between μ- and δ-opioid receptors in naïve animals has also been previously reported but is not understood (Porreca et al., 1987; Porreca et al., 1990). The demonstration of κ-mediated antianalgesia has been reported in rats and humans (Gear et al., 1999) and in vitro electrophysiological data describes a possible antagonistic mechanism (Pan et al., 1997). However, we do not know if this mechanism is responsible for the nucleus accumbens κ-mediated antianalgesia described here for the first time. Finally, the requirement of nucleus accumbens nicotinic receptors for opioid antinociception was demonstrated (Chapter 5); however, the role of nucleus accumbens opioid receptors in nicotine antinociception remains unknown. Given the demonstration that opioid receptors are required for nicotine antinociception (Aceto et al., 1993) it would be expected that nucleus accumbens opioid receptors would also play a role. Future investigations of these questions might not only provide insight into the interactions between the opioid receptor subtypes and nicotinic receptors but also might add to our knowledge regarding the clinical management of both acute-onset and morphine-resistant pain.

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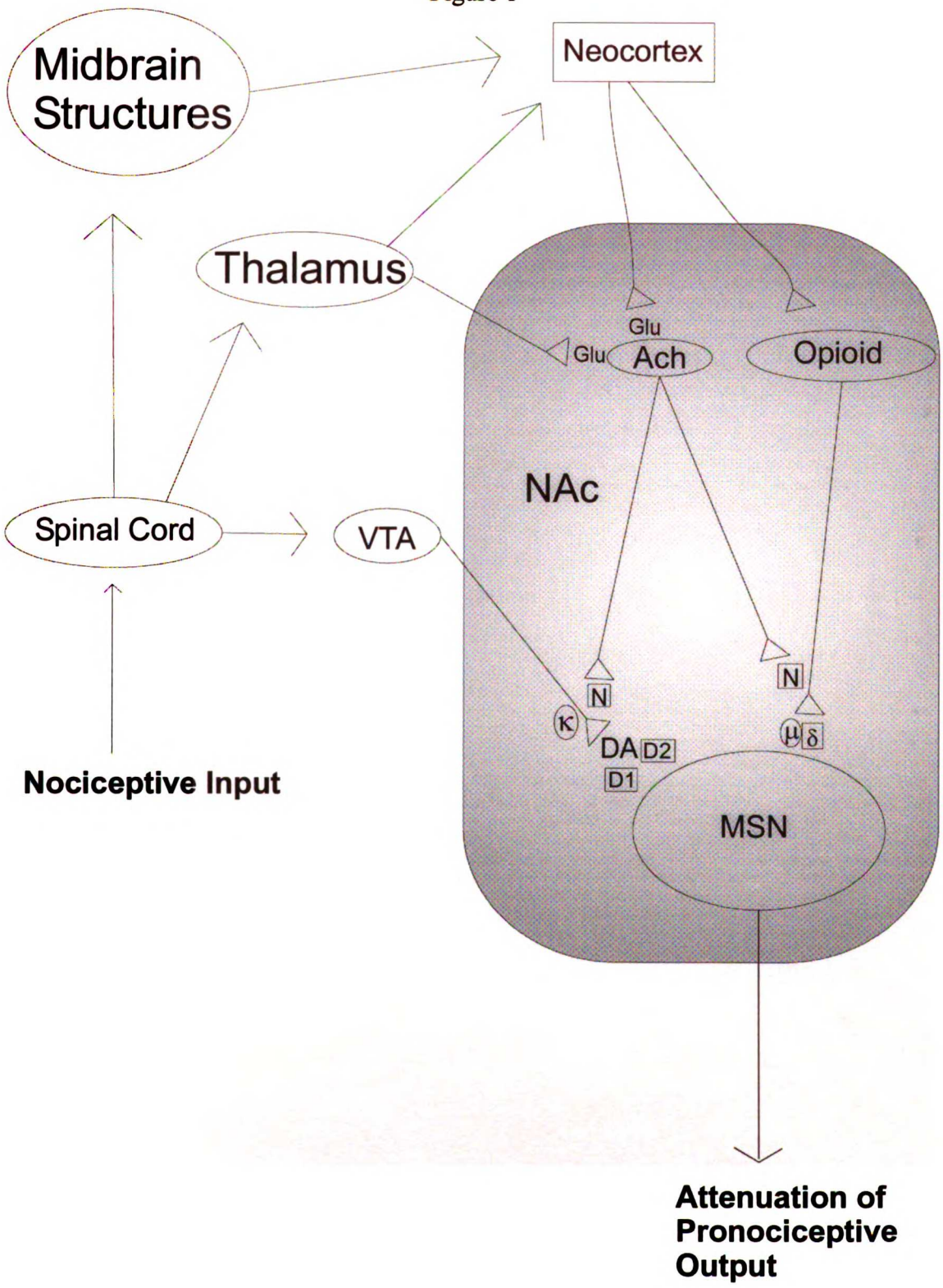
Table 1. Summary of experimental findings. Under the column heading "Antinociceptive effect of main treatment" the symbol "+" or "—" indicates antinociception or a lack of antinociception, respectively, following main treatment. Under the column heading "Intra-NAc modulation of antinociceptive effect" the symbol "—" indicates no effect on the antinociceptive effect of main treatment, while "X" indicates blockade of the antinociceptive effect of main treatment. The symbol "*" indicates a hyperalgesic effect.

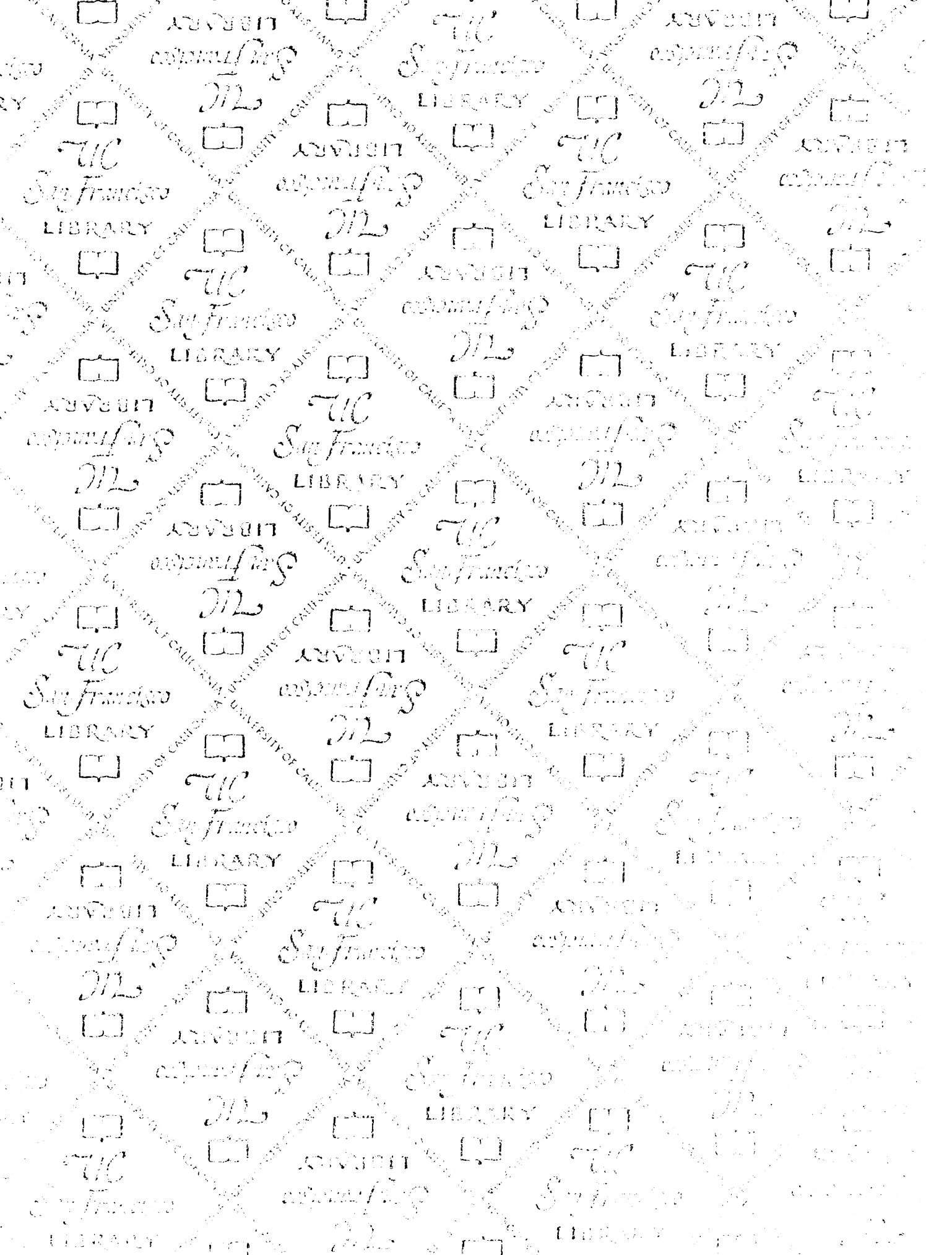
Table 1.

			Intra-NAc modulation of antinociceptive effect					
Physiologic state	**Main treatment**	**Antinociceptive effect of main treatment**	CTOP	naltrindole	*nor*-binaltorphimine	U69,593	mecamylamine	flupentixol
Naïve	none		—	—		—	—	—
	capsaicin	+	X	X	—	X	X	X
	morphine 10 mg/kg	+				—	—	
	morphine 5 mg/kg	+					X	
	DAMGO	—						
	DPDPE	—						
	DAMGO+DPDPE	+				X	X	
Morphine tolerant	none		—	—	—			—
	capsaicin	+	—	—	—		X	X
	morphine 10 mg/kg	—						
Morphine withdrawal	none		—	—				—
	capsaicin	+	X	—	—	—		
	morphine 10 mg/kg	—						
	DAMGO	+				X		
	DPDPE	—						
	DAMGO+DPDPE	+						
Nicotine tolerant	none						*	
	capsaicin	+					—	
	morphine 2.5 mg/kg	+					X	
	morphine 5 mg/kg	+					—	

Figure 1. Proposed diagram of nucleus accumbens antinociceptive circuitry. The shaded area represents nucleus accumbens (NAc) consisting of medium spiny neurons (MSN), cholinergic interneurons (Ach) and opioidergic neurons. Dopaminergic (DA) input from the ventral tegmental area (VTA) innervates the MSN population while glutamatergic (Glu) input from the neocortex innervates the cholinergic interneurons. Nicotinic receptors (N) presynaptically regulate DA and endogenous opioid release. Kappa-opioid (κ) receptors also presynaptically regulate DA. The response of MSN to dopaminergic (D1, D2) and opioidergic (μ, δ) stimulation is inhibition of pronociceptive output. Please see text for references.

Figure 1


Midbrain Structures
Neocortex
Thalamus
Glu
Glu
Ach
Opioid
NAc
Spinal Cord
VTA
N
κ
DA
D2
D1
N
μ
δ
MSN
Nociceptive Input
Attenuation of Pronociceptive Output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