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Physiological and pharmacological analysis of an ascending nociceptive control in the rat

Author
Gear, Robert Winfield

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Physiological and Pharmacological Analysis of an Ascending Nociceptive Control in the Rat

by

Robert Winfield Gear

DISSERTATION

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of the

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Dedication

This dissertation is dedicated, as is my career, to those who suffer from chronic pain. It is my hope that my work will contribute in some small way to the relief of their suffering.

Preface

As I put the final touches on this dissertation, I am aware, and a little self-conscious, that I am very likely one of the oldest students ever to graduate from UCSF. In these closing days of the twentieth century, more and more middle-aged people find themselves making fundamental changes in their careers. For many individuals the exigencies of the modern job market bring to bear difficult decisions and unwanted changes. Others, like myself, have had the good fortune to be presented with opportunities that have not always been available to people in mid-life.

Sometimes people kindly mention to me that they admire what I have accomplished. While I certainly appreciate the generosity of these well-intentioned individuals, I am constantly aware of how dependent I have been on the support of so many other people. This dissertation, for which I get the credit, certainly could not have existed without the funding contributed by the taxpayers of this country or the scientific achievements of those who have gone before me or the dialogues I have had with colleagues or the support of friends or, of course, the unbelievably generous mentorship of Jon Levine. With all of these resources miraculously available to me, all I had to do was do it.

To acknowledge every person who has contributed to this work would be impossible, but I am grateful to be able to thank at least of few of those to whom I am so profoundly indebted.

My oldest and deepest debt is, of course, to my parents, Winfield and Isabella Gear, who were born into an era when even undergraduate college education was rare for anyone without substantial family resources. Like so many people of their generation, my parents made their living in agriculture, and they

worked hard to make available to their children opportunities they themselves never enjoyed. But even though they lacked formal education, my parents never seemed to regret the life they had. Indeed, they demonstrated by example that how you live your life does not depend on how you make your living.

My wife, Billie Jean, is an exceedingly tolerant woman. She never said, "If leaving your dental practice and plunging us into financial catastrophe is what you want, I'll give you my blessing," but she might have made that statement; it wouldn't have been far from the truth. Her willingness to face financial disaster, however, did not extend to physical disaster: the Loma Prieta Earthquake struck one month after we moved to San Francisco in September, 1989. The very day we finally got rid of all the packing boxes and hung all the pictures was the day the earthquake turned our flat upside down. This event was a severe test of Billie's enthusiasm for graduate school. But ultimately, although she may have wavered a little, she never buckled, and her love and support have sustained me throughout. Without Billie and our two daughters, Gena and Heather, nothing would be worthwhile.

What can I say about Charles McNeill, D.D.S., my mentor in orofacial pain, my friend and my advocate? There is no question that I would not be in graduate school, could not have seen the possibilities, and would not have had the opportunities without his encouragement, guidance and support. Although I can acknowledge him and thank him, there is no way I will ever be able to repay him. Thank you, Chuck.

When I first entered the Graduate Program in Oral Biology, Caroline Damsky, Director of the Program, became my scientific mom and took great pains to ensure that my early graduate career would be successful. To her and to my graduate advisor, Peter Sargent, and to the Program in Oral Biology I owe nothing

less than my existence as a scientist. Without their moral and financial support, I could not have stayed the course.

Sara Ahlgren was the first graduate student in Jon Levine's lab, Michael Gold was the second, and I am the third. The first big obstacle to hurdle is the infamous qualifying oral examination which every Ph.D. student must take in order to advance to candidacy. I'll never forget how Sara and Mike took me under their wings and helped to prepare me for this examination. They became, in essence, my big sister and brother, which is an odd thing, since I'm old enough to be their father. And then Kimberly Tanner and, more recently, Holly Strausbaugh, came into the lab as graduate students. Although each student is immersed in her or his own research project, there is a camaraderie among us that sustains us when things are not working well and that enhances the pleasure we take in each other's successes. In particular I must mention the long conversations with Kimberly and Mike about my work. Without their insight and their perspective, I would have had a much harder time. I must add that these people stand in stark contrast to the general perception in this country that the quality of education, particularly in science, is in decline. These are brilliant students who offer great hope for the future of medicine and science in our country, and I count it a great privilege to have been associated with them.

I remember sometime in the early 1980's, when I was practicing dentistry in a small northern California town, that I saw a television program called "Nova" about pain and pain mechanisms. Jon Levine was featured on that program for his work on the placebo effect. I remember how fascinated I was with these findings. It never dawned on me then that I would ever actually be in the laboratory of this scientist. Even today, as I leave my house in the morning, I often say to Billie (in imitation of Dorothy and her friends in *The Wizard of Oz*), "I'm off to see the

wizards." It still amazes me that he took me on as a student. There is no way I will ever be able, with mere words, to acknowledge the debt I owe Jon Levine. He is my friend and whatever I accomplish as a scientist will always bear his imprint.

Some people think it takes courage to return to graduate school so late in life as I have done. I am not courageous. I am simply engaged in composing a life at the end of which the only things that will have mattered is that I took care of my obligations as best I could, I left behind a good family, and I enjoyed the journey.

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The text of Chapter 1 of this dissertation first appeared in *The Journal of Neuroscience.* The coauthor listed in this publication, Jon D. Levine, directed and supervised the research which forms the basis for this dissertation.

Abstract

Although spinally administered opioids are generally assumed to produce analgesia by inhibition of spinal nociceptive pathways, recent studies have suggested that supraspinal opioidergic mechanisms also contribute to their antinociceptive effect. To test the hypothesis that spinally administered opioids produce antinociception, in part, by an ascending circuit, the ability of spinal intrathecal (i.t.) administration of the μ-opioid agonist [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) to attenuate a nociceptive reflex at a distant (trigeminal) site was examined. I.t. DAMGO attenuated the jaw-opening reflex (JOR), and this effect was antagonized by naloxone injected into either the third cerebral ventricle (i.c.v.) or into the nucleus accumbens (NAC) but not into either the periaqueductal gray (PAG) or the rostral ventral medulla (RVM). I.t. lidocaine or spinal cord transection at the T_5-T_6 level also attenuated the JOR, and this attenuation was also sensitive to i.c.v. naloxone.

Examination of the opioid circuitry in NAC that mediates the ascending nociceptive control demonstrated that while injection of either Cys^2,Tyr^3,Orn^5,Pen^{7}amide (CTOP) or naltrindole (μ- or δ-antagonists, respectively) prevented attenuation of the JOR by i.t. DAMGO, administration of a combination of DAMGO plus the δ-agonist D-Pen^{2,5}-enkephalin (DPDPE), was needed to attenuate the JOR suggesting that antinociception mediated by NAC opioids is dependent on co-activation of μ- and δ-opioid receptors. Intra-accumbens injection of quaternary lidocaine (QX-314) also attenuated the JOR suggesting that antinociception results from inhibition of NAC efferent activity.

Involvement of the RVM in the ascending nociceptive control was examined by intra-RVM injection of either naloxone or the $GABA_A$ receptor

agonist muscimol. Muscimol, but not naloxone, blocked the antinociceptive effect of i.t. DAMGO, NAC μ-/δ-opioid combination or NAC lidocaine. In contrast, intra-RVM naloxone as well as muscimol blocked the antinociceptive effect of activation of the descending control by PAG DAMGO. Taken together, these results suggest that there exists an ascending nociceptive control that produces antinociception by inhibiting efferent activity from NAC; inhibition of this activity may de-facilitate pro-nociceptive RVM GABAergic activity. These findings provide the first direct evidence for the existence of an ascending antinociceptive control produced by spinal opioid administration, and suggest that this antinociceptive effect is mediated by NAC endogenous opioidergic and RVM GABAergic mechanisms.

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

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Introduction

The antinociceptive effect of systemically administered opioid analgesics is thought to result from action at CNS pain modulation circuits. Activation of these circuits has been accomplished by injection of opioids into specific CNS sites. For example, injection of morphine into the periaqueductal gray (PAG) has been proposed to produce antinociception by activation of a descending nociceptive control circuit that is relayed through the rostral ventral medulla (RVM) to the spinal cord (Basbaum and Fields 1978; Basbaum and Fields 1984; Fields and Basbaum 1994). Injection of morphine into RVM also produces antinociception as does spinal intrathecal (i.t.) morphine administration. Activation of the descending control by intra-PAG or intra-RVM opioid injection can be antagonized by injection of antagonists (e.g., naloxone) into the more caudal sites (Levine, Lane et al. 1982; Kiefel, Rossi et al. 1993). Until recently, the spinal cord was assumed to be the "endpoint" of this descending circuit; the role of the spinal mechanism was to gate signals in spinal nociceptive pathways. However, recent studies have reported that antinociception produced by the i.t. administration of opioids can be partially antagonized by intracerebroventricular (i.c.v.) administration of naloxone suggesting that supraspinal endogenous opioids contribute to the antinociceptive effect of the spinally administered opioid (Holmes and Fujimoto 1992; Miaskowski and Levine 1992). If so, this supraspinal contribution is presumably mediated by an ascending neural circuit. This dissertation describes research that tested the hypothesis that such an ascending nociceptive control circuit exists and, in addition, examined supraspinal mechanisms that mediate this circuit.

Although the descending nociceptive control is the most extensively studied of the CNS circuits implicated in pain modulation, other opioid-mediated pain modulation circuits have been proposed. For example, injection of opioids into many supraspinal sites that have not been identified specifically as components of the descending nociceptive control circuit produces antinociception (Yaksh, Yeung et al. 1976). A number of these sites, such as the amygdala (Helmstetter, Bellgowan et al. 1993) and the nucleus accumbens (NAC) (Dill and Costa 1977; Xuan, Shi et al. 1986; Yu and Han 1989; Yu and Han 1990; Yu and Han 1990; Tseng and Wang 1992) are important components of the limbic system, but the physiological role these sites play in nociception has not been well elucidated. In addition, a diffuse noxious inhibitory control (DNIC), which is activated by noxious stimulation, is inhibited by systemically administered naloxone (Le Bars, Chitour et al. 1980). Although DNIC is supraspinally mediated (Bouhassira, Villanueva et al. 1992), lesions of the RVM have no effect on DNIC (Bouhassira, Chitour et al. 1993) suggesting that there are distinct neural circuits that mediate the descending control and DNIC.

Review of studies suggesting ascending nociceptive modulation

An ascending circuit that mediates antinociception was proposed to explain the observations that antinociception produced by spinally administered calcium could be antagonized by naloxone administered either spinally (Welch, Stevens et al. 1992) or supraspinally (Lux, Welch et al. 1988) implicating the involvement of spinal as well as supraspinal endogenous opioids in mediating the antinociceptive properties of i.t. calcium. Further evidence that supraspinal endogenous opioids contribute to the antinociceptive effect of spinal opioids was reported by Miaskowski and Levine who demonstrated that antinociception produced by i.t. administration of the μ-opioid [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin

(DAMGO) is antagonized by an opioid antagonist administered i.c.v. in the rat (Miaskowski and Levine 1992). Finally, antinociception by spinal morphine was also shown to be antagonized by i.c.v. naloxone in mice (Holmes and Fujimoto 1992).

All of the studies cited above utilized tests of nociception that rely on reflexes mediated in the lumbar spinal cord where the spinal opioid is administered; that is, the site of nociceptive testing was not distant from the site of drug administration. Therefore, any observed antinociceptive effect would result from the local action of the spinal opioid as well as the putative supraspinal mechanism. Thus, the involvement of supraspinal endogenous opioids was inferred from the decrease in antinociception produced by the i.c.v. opioid antagonist, and was, therefore, based on indirect evidence. Indeed, one study suggested that the i.c.v. naloxone "activated" a descending antianalgesic pathway (Holmes and Fujimoto 1992) (see Chapter 2 for further discussion).

Other observations that spinal opioids produce supraspinally mediated antinociception was reported in a study of RVM "on-cells" and "off-cells" (for review, see (Fields, Heinricher et al. 1991). Morphine, administered i.t., produced changes in on- and off-cell activity that are similar to the changes in activity of these cells seen after administration of systemic morphine (Heinricher and Drasner 1991). Importantly, the spinal administration of the local anesthetic lidocaine produced similar effects. However, in this study it was not determined whether i.c.v. naloxone would antagonize the effects of the spinal administered drugs on RVM on-cells and off-cells; that is, the possible involvement of supraspinal endogenous opioids was not examined. Also, the nociceptive testing in this study utilized noxious thermal stimulation to the tail which precluded separation of the site of spinal drug application from the site of nociceptive testing.

Taken together, these studies suggest that the antinociception produced by spinal opioids results in part from the activation of a supraspinal antinociceptive mechanism, and this supraspinal contribution to antinociception appears to be mediated by endogenous opioids.

Major findings from the present research

In order to observe directly the contribution of supraspinal endogenous opioids to antinociception produced by spinally administered opioids, a trigeminal nociceptive model, the jaw-opening reflex (JOR), was chosen since it separates the site of drug administration (i.e., the lumbar spinal cord) from the site of the nociceptive reflex. My initial studies characterized the effects of time, anesthetic agent, and intensity and frequency of tooth pulp stimulation on the JOR in the rat. This characterization resulted in adoption of anesthetic and tooth pulp stimulation protocols (described in Chapter 1 of this dissertation) that produce stable electromyographic (EMG) recordings over a period of at least three hours. It has been reported that the JOR can be evoked by non-noxious as well as noxious stimuli. However, "there are no cases where the JOR is suppressed and pain is still experienced from tooth pulp shock; the suppression of the JOR may therefore be an accurate index of analgesia. However, in humans treatments that produce analgesia have not been shown to produce suppression of the JOR. Thus, the JOR that persists following analgesia treatments is not a reliable index of either analgesia or pain" (Mason, Strassman et al. 1985). In light of these considerations, I chose to determine antinociceptive effect by calculating percent attenuation of the JOR. I characterized the response of the JOR EMG to administration of opioids (either systemically administered morphine or i.c.v. DAMGO). These studies revealed that the JOR EMG resulting from tooth pulp stimulation intensities at or near three times the threshold, as well as being stable over time,

are reproducibly sensitive to opioid-induced antinociception; furthermore, stimulation intensities in this range typically evoke maximal amplitude of the JOR EMG.

Using the JOR in the rat, I have demonstrated that opioids administered to the lumbar spinal cord evoke antinociception mediated by an opioidergic link in the NAC. This effect appears to depend on inhibition of tonic activity in spino-supraspinal pathways that presumably arises as "spontaneous" activity (i.e., neuronal activity recorded in the absence of stimulation) in dorsal horn neurons (Handwerker, Iggo et al. 1975; Cervero, Molony et al. 1977; Menetrey, Giesler et al. 1977). (The activation of thalamic neurons by spontaneous activity arising in the spinal cord has been reported demonstrating that this activity ascends through spino-supraspinal tracts (Kenshalo, Giesler et al. 1980).) Therefore, I refer to this circuit as an "ascending nociceptive control." Examination of NAC mechanisms revealed that NAC opioids produce antinociception by suppression of NAC efferent activity. Finally, this research shows that the ascending nociceptive control is mediated in the RVM by a different mechanism than that which mediates the descending nociceptive control.

Conclusions

The results of the studies presented in this dissertation emphasize the participation of the spinal cord in an ascending circuit that regulates nociception. This regulatory circuit involves interactions between the spinal cord, the NAC, and the RVM, and, when considered in the context of other CNS pain modulation circuits, implies that the spinal cord not only serves to effect nociceptive modulation according to signals it receives from the brainstem, but also serves to affect nociception by sending modulatory inputs to structures normally associated with the limbic system and the descending nociceptive controls.

Activity in spinal projection neurons of the ascending control circuit increases nociception, suggesting that this circuit is part of a pro-nociceptive system. Whereas the descending control functions to increase antinociception, the ascending control may function to increase nociception. The two systems are coordinated by opioidergic circuits that turn the descending control on by disinhibition of antinociceptive signals and turn the ascending control off by inhibition of pronociceptive signals.

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Chapter 1

Antinociception Produced by an Ascending Spino-Supraspinal Pathway

Abstract

Studies in mice and rats have shown that antinociception produced by intrathecal (i.t.) administration of opioids can be partially inhibited by intracerebroventricular (i.c.v.) administration of naloxone. In this study we tested the hypothesis that this inhibition by i.c.v. naloxone results from antagonism of supraspinal endogenous opioid-mediated antinociception produced by the action of i.t. opioids on an ascending antinociceptive pathway. In rats lightly anesthetized with urethane/alpha-chloralose, i.t. DAMGO, i.t. lidocaine, or spinal transection at T_5-T_6 all attenuated the trigeminal jaw opening reflex (JOR) (i.e., were antinociceptive), an effect that was antagonized in each case by i.c.v. naloxone. These findings support the suggestion that there exists a pathway that ascends from the spinal cord to a supraspinal site that tonically inhibits antinociception mediated by supraspinal opioids. When activity in this ascending pathway is suppressed (e.g., by i.t. opioids or local anesthetics or by spinal cord transection), antinociception mediated by supraspinal opioids is disinhibited.

To determine the supraspinal site(s) at which endogenous opioid-dependent antinociception is evoked by i.t. opioids, we microinjected naloxone methiodide into several supraspinal sites. Microinjection of naloxone methiodide into nucleus accumbens but not into the rostral ventral medulla (RVM) or the periaqueductal gray matter (PAG) antagonized the suppression of the JOR produced by i.t. DAMGO or lidocaine. The possibility that this ascending pathway may represent

a source of spinal input to mesolimbic circuitry involved in setting the state of sensorimotor reactivity to noxious stimuli is discussed.

Introduction

A large body of research has implicated endogenous opioids in the modulation of pain at three principal CNS sites, the periaqueductal gray (PAG), the rostral ventral medulla (RVM), and the dorsal horn of the spinal cord. Since electrical stimulation or microinjection of morphine into more rostral sites (i.e., the PAG or RVM) increases the thresholds of spinal nociceptive reflexes, it has been suggested that this circuit functions as a descending antinociceptive control (Basbaum and Fields 1978; Basbaum and Fields 1984; Fields and Basbaum 1994). Recent studies, however, provide evidence that there is also an ascending (i.e., a spino-supraspinal) antinociceptive pathway through which spinal opioids evoke antinociception mediated by endogenous opioids at supraspinal site(s) (Fig. 1A). For example, exogenous (Holmes and Fujimoto 1992; Miaskowski and Levine 1992) or endogenous (Welch, Stevens et al. 1992) but also see (Lux, Welch et al. 1988) spinal opioids produce antinociception that can be partially antagonized by intracerebroventricular (i.c.v.) naloxone. However, these observations can be explained by alternative interpretations. For example, it has been proposed that i.c.v. naloxone "activates" a descending anti-analgesia system that antagonizes the antinociceptive action of i.t. opioids at the level of the spinal cord (Holmes and Fujimoto 1992) (Fig. 1B). Therefore, to determine if spinally administered opioids can act via an ascending spino-supraspinal circuit to produce analgesia, we devised an experimental model that separates the site of reflex measurement from the spinal site of opioid administration. In the lightly anesthetized rat, we measured the effect of intrathecally administered [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) on the amplitude of the jaw opening reflex (JOR) with or without naloxone administered into the third cerebral ventricle (i.c.v. naloxone). To further characterize the ascending pathway (i.e., whether it must be activated in

order to evoke antinociception mediated by supraspinal opioids, or if its activity must be suppressed in order to disinhibit supraspinal opioid-mediated antinociception), the effects on the JOR of i.t. lidocaine or spinal transection with or without i.c.v. naloxone were compared. Finally, to determine the supraspinal site at which naloxone acts to antagonize antinociception produced by i.t. DAMGO, we studied the effect of naloxone methiodide microinjected into several supraspinal sites on the ability of i.t. DAMGO to attenuate the JOR. Some of the results of this study have been previously reported in abstract form (Gear and Levine 1994).

Materials and Methods

The experiments were performed on 250 - 450 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) that were lightly anesthetized by intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma, St. Louis, MO), and 10 mg/kg methohexital (Brevital) for rapid induction of anesthesia. We chose urethane/α-chloralose for anesthesia because in preliminary experiments this anesthetic, unlike a single dose of pentobarbital or continuous infusion of methohexital, provided a stable JOR EMG (see below) over the time period (at least three hours) required to complete the experiments (data not shown). The animals were sacrificed by overdose of pentobarbital unless it was necessary to section their brains for histological purposes, in which case we sacrificed the animals by intra-cardiac perfusion of 4% formalin after insuring that they were in a deep state of anesthesia with pentobarbital.

Cannulation. To administer drugs to the area of the lumbar enlargement of the spinal cord, an i.t. catheter (PE-10; 10 μl volume) was inserted 8.5 cm caudally into the subarachnoid space through a slit in the atlanto-occipital membrane (Yaksh and Rudy 1976). It was not possible to determine by pharmacological/behavioral methods whether the catheters were correctly placed or whether any of the catheter placements would have resulted in motor deficits because the animals do not recover from urethane/α-chloralose anesthesia. However, in many experiments we checked catheter placement by injecting Evans blue dye and performing a post-mortem laminectomy to determine the location of the dye and the tip of the catheter. In all cases we observed that the position of the catheter varied only in its dorso-ventral relationship to the cord. That is, the tip of the catheter was sometimes positioned more dorsally to the cord and sometimes it was positioned more ventrally, but the catheter never turned back on itself or

perforated the dura. Also, while animals free of motor deficits would have been essential if we were measuring lumbar spinal reflexes which depend upon intact motor circuitry in the spinal cord (e.g., paw-withdrawal or tail-flick), in this study we employed the supraspinally mediated JOR.

To administer drugs to the third cerebral ventricle, an i.c.v. guide cannula (22 gauge) was positioned to allow drug delivery via insertion of a 30 gauge injection cannula. At the conclusion of the experiments i.c.v. sites were verified by injection of Evans blue dye, equal in volume to the drug injection, after which the brains were removed, sectioned and examined for dye location. In some experiments 25 gauge guide cannulae were positioned to allow microinjections via insertion of a 33 gauge injection cannula into specific supraspinal sites. These injection sites were verified by histological examination (70 μm sections stained with cresyl violet acetate) (Fig. 8 and Fig. 9).

Spinal transection. In experiments in which the spinal cord was transected, a laminectomy was performed after implantation of the i.c.v. cannula and the stimulating and recording electrodes for the JOR. The spinal cord was exposed, but not sectioned, by the removal of the dorsal portions of the T_5 and T_6 vertebrae. Spinal transection was performed after baseline JOR recordings and administration of i.c.v. naloxone (or vehicle) as described in "Results". These animals did not receive an i.t. catheter.

Jaw opening reflex. A bipolar stimulating electrode, fabricated from two insulated single-stranded 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 20 mm from the incisal edge of the tooth to the tip of the longest wire (Toda, Iriki et al. 1981). Access to the pulp of the incisor was through an opening in the labial surface of the tooth starting 2 mm

below the gingival crest and extending 4 mm toward the incisal edge. Dental composite resin held the electrode in place and sealed the opening in the tooth. 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 digastric muscle ipsilateral to the implanted tooth sufficiently deep to completely submerge the uninsulated end of the wire. A 22 gauge needle was inserted in the skin ventral to the midline of the mandible and connected to the ground terminal of the amplifier. Tooth pulp was stimulated with 0.2 ms square wave pulses at 0.33 Hz. Stimulation voltage was set at 3 times the threshold voltage for evoking the JOR EMG. Twelve consecutive evoked EMG signals were averaged per recording (Fig. 2).

Antinociception was measured as the percentage decrease (mean ± s.e.m.) from the average amplitude of three baseline recordings taken at five minute intervals. ANOVA and either the Student-Neuman-Keuls (SNK) test or the Fisher's test (Fisher 1949), as appropriate, were used to compare groups for significant differences ($p \leq 0.05$).

Drugs. Lidocaine (4% Xylocaine, Astra Pharmaceutical Products, Westborough, MA) without epinephrine was used as supplied by the manufacturer. [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and naloxone (Sigma, St. Louis, MO), and naloxone methiodide (Research Biochemicals International, Natick, MA), a quaternary derivative of naloxone, which has been shown to spread from the site of injection more slowly than naloxone (Schroeder, Weinger et al. 1991), were dissolved in physiological saline (0.9%), or artificial CSF (Leviel, Gobert et al. 1989). To retard rostral flow of the i.t. drug or vehicle, all animals were placed in a prone position on an inclined surface (approximately 30 degrees) with the head higher than the tail. I.t. drug or vehicle volumes were 15 µl

followed by 10 μl of vehicle (equal to the volume of the i.t. catheter). I.c.v. injection volumes were 1 μl. Microinjections into specific supraspinal sites were carried out over a period of 90 seconds, and the injection cannulae were left in place an additional 30 seconds after injection.

Results

Intrathecal DAMGO

To determine if i.t. opioids modulate nociception via an ascending pathway, the effect of i.t. DAMGO (75 ng - 7.5 μg) on the JOR, a reflex which should not be affected directly by i.t. drugs, was determined. Figure 2 illustrates an example of the JOR EMG waveform before (Fig. 2A) and 15 minutes after (Fig. 2B) the administration of i.t. DAMGO (7.5 μg). DAMGO produced a dose-dependent suppression of the JOR (Fig. 3). I.t. vehicle had no significant effect on the JOR.

To control for systemic absorption of i.t. DAMGO, the highest dose of DAMGO (7.5 μg) was also administered intravenously in a different group of animals ($n = 4$). Fifteen minutes after receiving this treatment the JOR amplitude recorded from this group was not significantly different from baseline ($-3 \pm 10\%$, mean ± s.e.m., $p > 0.05$).

To determine if attenuation of the JOR by i.t. DAMGO is dependent on supraspinal opioids, i.c.v. naloxone (2 μg) was administered one minute before i.t. DAMGO (7.5 μg), and the JOR was recorded 15 minutes later. In the presence of naloxone (i.c.v.), DAMGO (i.t.) failed to significantly suppress the JOR ($p < 0.05$). To determine if i.c.v. naloxone might exert an independent (i.e., hyperalgesic) effect, naloxone was administered i.c.v. with i.t. vehicle. This treatment did not significantly affect JOR amplitude (Fig. 3) ($p > 0.05$).

Intrathecal lidocaine

Opioid-mediated antinociception at the supraspinal site could be produced either by an excitatory action or by disinhibition. If disinhibition is the mechanism, the ascending pathway must be tonically active and the suppression of this tonic activity by a spinally-administered local anesthetic should mimic the ability of i.t. DAMGO to suppress the JOR. Therefore, lidocaine (0.6 mg) was

administered i.t. Fifteen minutes after i.t. administration, lidocaine suppressed the mean JOR amplitude 52% below baseline (Fig. 3). Suppression of the JOR by i.t. lidocaine or i.t. DAMGO was not significantly different ($p > 0.05$). These results suggest that the ascending pathway is tonically active and must be suppressed in order to attenuate the JOR.

Since lidocaine might enter the general circulation following i.t. administration and act at a site other than the lumbar spinal cord, the same dose of lidocaine was administered subcutaneously at the nape of the neck in a separate group of animals ($n = 6$). Fifteen minutes after receiving this treatment the JOR amplitude recorded from this group was not significantly different from baseline ($+4.2 \pm 6.94\%$, mean ± s.e.m., $p > 0.05$).

To determine if attenuation of the JOR by i.t. lidocaine is mediated by a supraspinal opioidergic mechanism similar to that mediating the action of i.t. DAMGO, i.c.v. naloxone (2 μg) was administered one minute before i.t. lidocaine (0.6 mg) and the JOR was recorded 15 minutes later. Naloxone completely blocked the ability of lidocaine to attenuate the JOR ($p < 0.05$) (Fig. 3), strongly suggesting that i.t. lidocaine, similar to i.t. DAMGO, attenuates the JOR by a supraspinal opioidergic mechanism.

Spinal transection

If the ascending antinociceptive system is tonically active, as suggested by the effects of i.t. lidocaine, other methods of suppression of activity in ascending pathways should also produce antinociception/inhibition of the JOR that is antagonized by i.c.v. naloxone. Therefore, we next performed spinal transection at the T_5-T_6 level. To determine if a supraspinal opioidergic mechanism is involved, spinal transection was performed in the presence of either naloxone or vehicle administered i.c.v. The following protocol was performed: 1) JOR baseline

measurements were recorded in acutely laminectomized animals, 2) i.c.v. naloxone (2 μg) or vehicle was administered, 3) 5 minutes later the spinal cord was transected, 4) JOR was measured at 15 minute intervals for one hour. (Although acute spinal transection can lead to hyperresponsiveness of spinal reflexes, in our experiments the JOR did not demonstrate hyperresponsiveness.) The group receiving i.c.v. vehicle showed significant JOR attenuation compared to the group receiving i.c.v. naloxone (Fig. 4) ($p < 0.05$). I.c.v. naloxone almost completely blocked the ability of spinalization to attenuate the JOR suggesting that this attenuation is mediated by a supraspinal opioidergic mechanism similar to that mediating the effects of intrathecally administered lidocaine or DAMGO.

Supraspinal sites

To locate the supraspinal site at which endogenous opioids contribute to the ascending antinociception produced by i.t. DAMGO, we first evaluated the effect of the injection of naloxone methiodide into the PAG and the RVM, two supraspinal sites that contribute to opioid analgesia in descending antinociceptive systems. Ventrolateral PAG sites were chosen because of previous reports implicating these sites in morphine antinociception (Yeung, Yaksh et al. 1977; Yaksh, Al-Rhodhan et al. 1988) (sites plotted in Fig. 8). Naloxone methiodide is a quaternary derivative of naloxone chosen because it spreads more slowly than naloxone (Schroeder, Weinger et al. 1991). I.t. DAMGO significantly attenuated the JOR in the groups of rats receiving naloxone methiodide microinjected into RVM or PAG as compared to the group receiving i.c.v. naloxone methiodide (Fig. 5). Thus, the opioidergic mechanisms in RVM or PAG do not appear to be required in order to observe the antinociceptive effect of i.t. DAMGO. Since opioids microinjected into a number of supraspinal sites have been shown to be antinociceptive (Yaksh, et al., 1976), we tested the ability of bilateral

microinjections of naloxone methiodide into several of these sites to antagonize DAMGO (i.t.) suppression of the JOR. Microinjection of naloxone methiodide, but not vehicle, into nucleus accumbens not only blocked suppression of the JOR by i.t. administration of DAMGO (7.5 μg), but showed significant overshoot suggesting a hyperalgesic state ($p < 0.05$) (Fig. 6). The specificity of the nucleus accumbens as a site at which opioid-dependent antinociception is evoked by i.t. DAMGO was further confirmed by the observation that microinjection of naloxone methiodide into sites surrounding nucleus accumbens failed to antagonize suppression of the JOR by i.t. DAMGO (Fig. 6, Fig. 9). The groups receiving i.t. DAMGO and either "offsite" injection of naloxone or "onsite" injection of CSF were not significantly different from each other ($p > 0.05$), but were significantly different from either of the groups receiving "onsite" injection of naloxone ($p < 0.05$). Finally, preliminary findings indicate that naloxone methiodide had no effect when microinjected bilaterally into the habenula, or amygdala (data not shown).

To confirm that nucleus accumbens is also the site of the opioid link mediating suppression of the JOR by i.t. lidocaine, we tested the ability of bilateral microinjections of naloxone methiodide to block suppression of the JOR by i.t. lidocaine. Naloxone methiodide microinjected into nucleus accumbens, but not into surrounding sites, blocked suppression of the JOR by i.t. lidocaine ($p < 0.05$) (Fig. 7, Fig. 9). These findings confirm that an opioid link in nucleus accumbens mediates suppression of the JOR by either i.t. lidocaine or i.t. DAMGO, and also confirm that the ascending pathway is tonically active and must be suppressed in order to disinhibit this opioid link in nucleus accumbens.

Discussion

Spinally-evoked antinociception mediated by supraspinal opioids

In this study we demonstrate in the lightly anesthetized rat the existence of a pathway that ascends from the spinal cord to a supraspinal site that produces antinociception mediated by supraspinal opioidergic mechanisms by showing that: 1) the JOR is suppressed by DAMGO or lidocaine administered intrathecally at the lumbar level of the spinal cord, or by spinal transection, and 2) that supraspinally administered naloxone (i.e., i.c.v. or in nucleus accumbens) antagonizes this effect. The observation that i.t. lidocaine or spinal transection mimics i.t. DAMGO in suppressing the JOR suggests that the ascending pathway is tonically active. We propose that inhibition of activity in this ascending pathway by spinal analgesic agents (i.e., opioids or local anesthetics) disinhibits supraspinal opioid-mediated antinociception. This supraspinal antinociceptive mechanism appears to have a global effect on nociceptive reflexes as it has been detected at the site of lumbar drug administration (Holmes and Fujimoto 1992; Miaskowski and Levine 1992) as well as a trigeminal nociceptive reflex (i.e., the JOR). Also, the reported observation that morphine, administered intrathecally to the lumbar spinal cord, is effective in the treatment of head and neck cancer pain (Andersen, Cohen et al. 1991) suggests that the ascending pathway may be relevant to the treatment of pain. In addition, a number of studies have reported that spinally administered local anesthetics potentiate the antinociceptive effects of spinal morphine (Akerman, Arwestrom et al. 1988; Penning and Yaksh 1990; Maves and Gebhart 1992). Given our current findings, it is possible that this potentiation is mediated by suppression of activity in the ascending pathway which disinhibits a supraspinal opioidergic mechanism. At present, nothing is known of the physiological conditions under which antinociception mediated by inhibition

of the ascending pathway might occur. Since endogenous opioids are released in the spinal cord under various conditions (Watkins, Cobelli et al. 1982; Yaksh, Terenius et al. 1983; Chung, Fang et al. 1984; Cesselin, Le Bars et al. 1985; Le Bars, Bourgoin et al. 1987; Bourgoin, Le Bars et al. 1990; Taylor, Pettit et al. 1990), and since we have shown that intrathecal opioids produce antinociception via the ascending pathway, we suggest that endogenously released spinal opioids might act on the ascending pathway in a similar manner.

Site of action of supraspinal naloxone

In this study we demonstrate that microinjection of naloxone methiodide into the nucleus accumbens, but not into several other supraspinal sites (i.e., RVM, PAG, or sites adjacent to nucleus accumbens), blocks the suppression of the JOR by either i.t. DAMGO or i.t. lidocaine. These results suggest that nucleus accumbens contains an opioidergic mechanism important in mediating the antinociceptive effect of i.t. DAMGO as well as i.t. lidocaine, and further suggests that this opioid mechanism is activated by disinhibition (i.e., suppression of tonic activity in the ascending pathway). In support of a role for the nucleus accumbens in processing nociceptive information, several investigators have reported that microinjection of morphine into nucleus accumbens produces antinociception (Dill and Costa 1977; Jin, Zhou et al. 1986; Yu and Han 1990). Furthermore, nucleus accumbens contains opioid receptors (Atweh and Kuhar 1977; Stein, Hiller et al. 1992), and is immunoreactive for both met-enkephalin and β-endorphin (Hong, Yang et al. 1977; Ma and Han 1991; Ma, Shi et al. 1992). Of note, Han and colleagues have proposed the existence of a "mesolimbic loop of analgesia" in which the opioid circuitry in the nucleus accumbens plays an important role (Han and Xuan 1986; Xuan, Shi et al. 1986; Yu and Han 1990; Ma and Han 1991; Ma, Shi et al. 1992; Ma, Shi et al. 1992). Spinal neurons which carry nociceptive

information have been shown to project directly to nucleus accumbens and other limbic structures (Burstein, Cliffer et al. 1987; Burstein and Giesler 1989; Cliffer, Burstein et al. 1991).

Diffuse noxious inhibitory controls (DNIC)

Antinociception produced via the ascending pathway appears to resemble DNIC in that an event remote from the site of application of a noxious stimulus is capable of raising the threshold of response to that stimulus (see (Le Bars, Villanueva et al. 1992) for review of DNIC). The antinociception produced by the ascending pathway and that produced by DNIC are, however, likely to result from different mechanisms since DNIC is mediated by excitatory activity in ascending pathways (Le Bars and Villanueva 1988; Villanueva, Bouhassira et al. 1988), whereas we demonstrate that inhibition of tonic activity in ascending pathways evokes antinociception.

Relevance to awake, pain-free state

Since, in our experiments, we used lightly anesthetized animals in an acute preparation, it is possible that animals in this state could be exhibiting a phenomenon not present in animals in an awake, pain-free state. However, a strong argument against this is that antinociception produced by spinally administered opioids is also antagonized by supraspinal opioid antagonists in awake, pain-free animals (Holmes and Fujimoto 1992; Miaskowski and Levine 1992). Nevertheless, it is important that our findings be confirmed in other experimental paradigms which avoid the use of anesthesia and procedures which stimulate nociceptors.

In summary, we demonstrate that either a spinally administered opioid (DAMGO) or a spinally administered local anesthetic (lidocaine) attenuates the trigeminal JOR and that in either case this attenuation is blocked by the

administration of naloxone methiodide into the nucleus accumbens. Spinal transection also suppress the JOR in a manner sensitive to supraspinal naloxone. These observations support the suggestion that suppression of tonic activity in an ascending pathway disinhibits a supraspinal antinociceptive circuit with an opioid link in nucleus accumbens (Fig. 1A). This inhibition of supraspinal opioid-dependent antinociception by ascending tonic activity implies that the net effect of spinal input into the limbic system is to facilitate nociceptive sensitivity. This facilitation may be suppressed by events that evoke the release of spinal endogenous opioids.

Figures

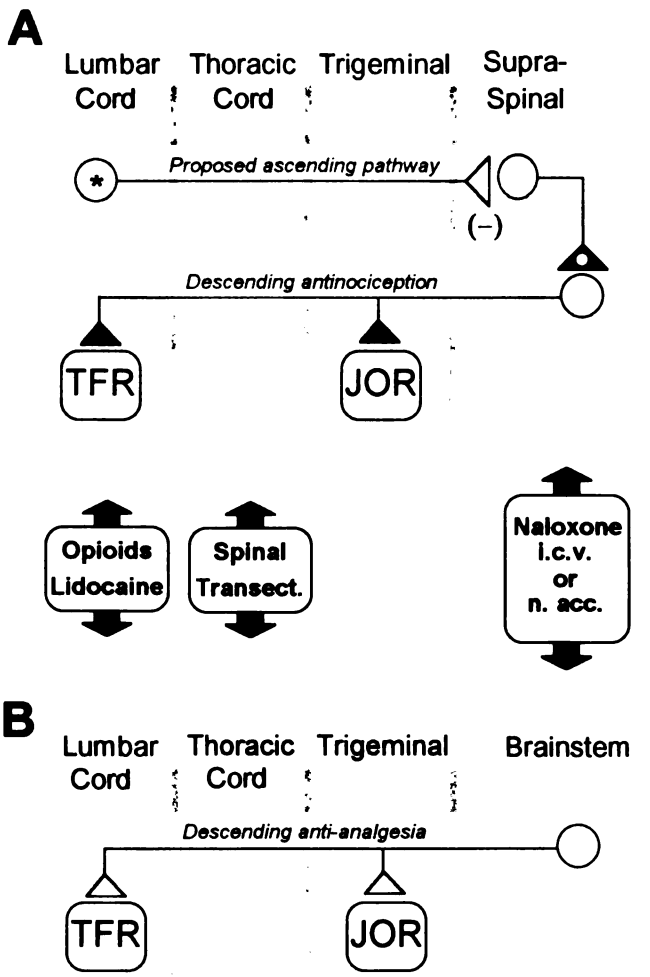
Figure 1.

A. Schematic illustration of the proposed ascending antinociceptive pathway. The asterisk indicates the tonically active ascending limb of the pathway that inhibits supraspinal opioidergic neurons (thereby enhancing nociceptive behavior) in nucleus accumbens. Suppression of activity in this ascending pathway by lumbar (i.t.) opioids or lidocaine, or thoracic spinal transection results in supraspinal opioid disinhibition which modulates spinal and trigeminal nociceptive reflexes as indicated by the filled triangles. Note that spinal analgesic agents (opioids or lidocaine), applied to the lumbar cord, act through two mechanisms by: 1) directly on the local synapses mediating the tail-flick reflex (TFR), and 2) suppressing the tonically active ascending pathway. Since the JOR is mediated by neuronal circuits located at a site distant from the lumbar cord, drugs applied to the lumbar cord can modulate the JOR only via an ascending pathway. Spinal transection at the thoracic cord also modulates nociception via the ascending pathway, however, this can only be observed in the JOR (or other supraspinally-mediated nociceptive reflexes) since reflexes mediated at the lumbar cord are disconnected from supraspinal influence.

B. Schematic illustration of a descending anti-analgesia circuit (Holmes and Fujimoto 1992). In this model opioids or lidocaine applied to the lumbar cord would only affect lumbar spinal reflexes (e.g., the TFR) by local action. The JOR is not affected since this model does not propose an ascending pathway. When i.c.v. naloxone is administered, anti-analgesic circuits are activated in the spinal cord (open triangles), but since i.c.v. naloxone alone has no effect on nociceptive thresholds, these substances act only to antagonize the action of endogenous or exogenous spinal opioids. Since the opioids in our experiments are administered

to the lumbar region of the spinal cord, the anti-analgesic substances have no effect on the trigeminal JOR. Also, these anti-analgesic substances are not proposed to antagonize the antinociceptive action of lidocaine. Spinal transection has no effect on the JOR in this model.

Figure 1



= Inhibits nociceptive behavior

= Inhibits nociceptive behavior (opioid)

= Enhances nociceptive behavior

* = Tonically active (–) = Inhibitory synapse

TFR = Tail flick reflex JOR = Jaw opening reflex

n. acc. = nuc. accumbens i.c.v = intracerebroventricular

Figure 2.

Examples of JOR EMG recordings. Tracings represent the average EMG in response to 12 tooth pulp stimuli. The JOR EMG response occurs with a latency of approximately 7-9 ms after tooth pulp stimulation shown as the downward stimulus artifact at the beginning of the sweep in panel A. The peak-to-peak distance (in mV) of the EMG signal was taken to be the magnitude of the EMG. A. A typical baseline EMG recording. Amplitude: 5.57 millivolts. B. Average EMG response 15 minutes after the administration of i.t. DAMGO (7.5 μg). Amplitude: 2.91 millivolts. In this example the percent decrease (i.e., "JOR suppression") is 48%. Formula: ((A - B) / A) x 100). Baseline values used in calculating the results of the experiments were based on 3 pre-treatment recordings (12 stimuli each) taken five minutes apart.

Figure 2

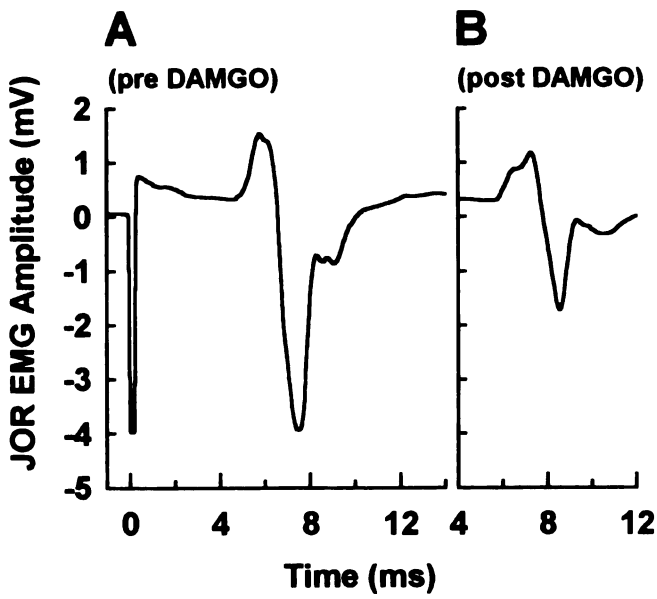

A
(pre DAMGO)
B
(post DAMGO)
JOR EMG Amplitude (mV)
2
1
0
-1
-2
-3
-4
-5
0
4
8
12
4
8
12
Time (ms)

Figure 3.

The effect of i.t. DAMGO, i.t. lidocaine (lido), or i.t. vehicle (veh) with or without i.c.v. naloxone (nlx) on the amplitude of the JOR. The legend beneath the graph indicates the i.t. treatment, the i.c.v. treatment and the number of animals in each experimental group. DAMGO (i.t.) dose-dependently attenuated the JOR. I.t. lidocaine also showed significant suppression of the JOR. The groups receiving lidocaine or the highest dose of DAMGO (without naloxone) were not significantly different from each other ($p > 0.05$) but were significantly different from the groups receiving i.t. vehicle/i.c.v. vehicle, i.t. vehicle/i.c.v. naloxone, or i.t. DAMGO/i.c.v. naloxone ($p < 0.05$); these last three group were not significantly different from each other ($p > 0.05$). In this and subsequent figures error bars indicate s.e.m.

Figure 3

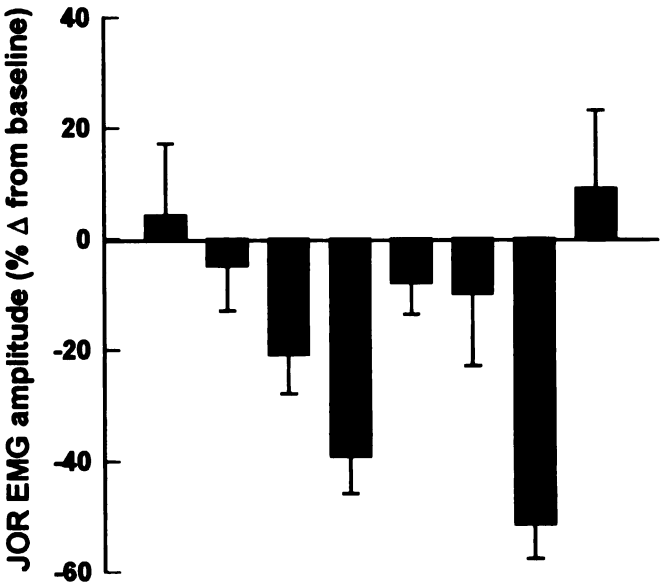

JOR EMG amplitude (% Δ from baseline)
40
20
0
-20
-40
-60
DAMGO (μg)
i.t. veh .075 .75 7.5 7.5 veh lido lido
i.c.v. veh nlx nlx nlx
n 5 5 6 14 14 5 14 6

Figure 4.

The effect of spinal transection on the JOR with or without i.c.v. naloxone. Spinal transection (transect.) in the presence of i.c.v. vehicle (veh) (circles, $n = 4$) suppressed the JOR, but spinal transection in the presence of i.c.v. naloxone (nlx) (squares, $n = 4$) remained near baseline levels, a significant difference ($p < 0.05$).

Figure 4

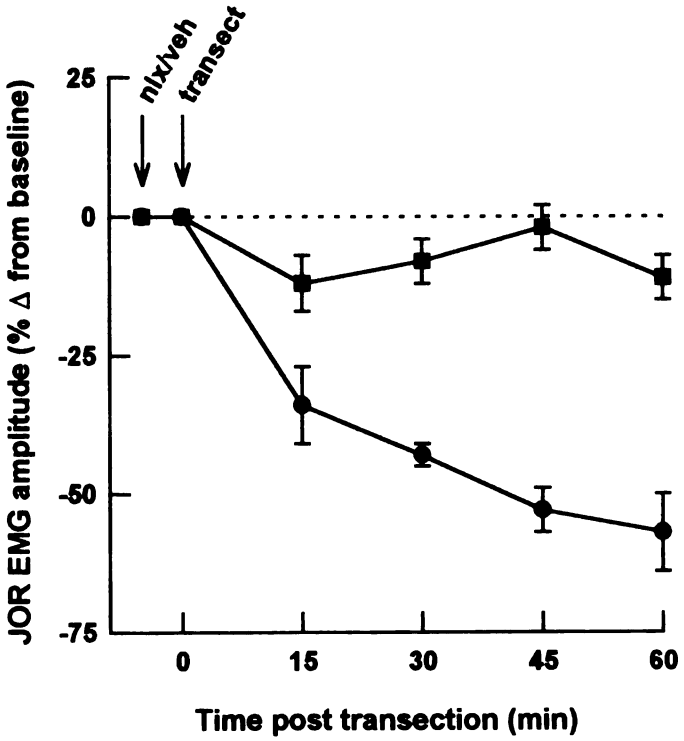

nlx/veh
transect
25
0
-25
-50
-75
JOR EMG amplitude (% Δ from baseline)
0
15
30
45
60
Time post transection (min)

Figure 5.

The effect of naloxone methiodide injected i.c.v. or microinjected in RVM or PAG. All groups received i.t. DAMGO. Only the group receiving i.c.v. naloxone methiodide (circles, n = 6) failed to show attenuation of the JOR. The groups receiving naloxone methiodide microinjected into either RVM (1 μg in 0.5 μl/side, squares, n = 6, sites plotted in Fig. 7B) or PAG (2 μg in 0.5 μl, triangles, n = 6, sites plotted in Fig. 7A) were significantly different from the group receiving i.c.v. naloxone methiodide ($p < 0.05$), but were not significantly different from each other ($p > 0.05$).

Figure 5

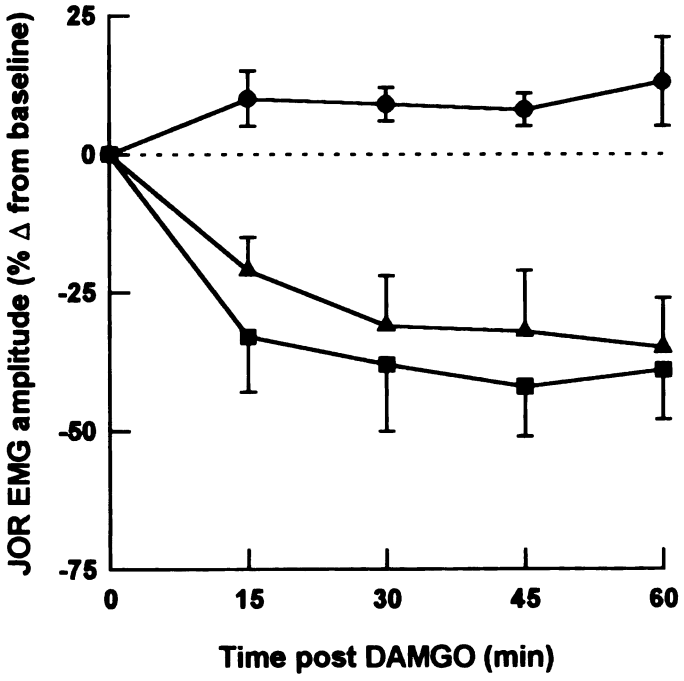

25
0
-25
-50
-75
JOR EMG amplitude (% Δ from baseline)
0
15
30
45
60
Time post DAMGO (min)

Figure 6.

The effect of i.t. DAMGO on the JOR with or without naloxone methiodide microinjected into specific rostral sites. Naloxone methiodide (1 μg in 0.2 μl CSF/side, circles, n = 6, sites plotted in Fig. 8A), but not vehicle (0.2 μl CSF/side, squares, n = 6, sites plotted in Fig. 8C), microinjected into nucleus accumbens five minutes before i.t. DAMGO prevented suppression of the JOR. Naloxone methiodide (1 μg in 0.2 μl CSF/side, upward triangles, n = 5, sites plotted in Fig. 8D) administered alone to nucleus accumbens had no effect on the JOR. Offsite injections of naloxone methiodide (1 μg in 0.2 μl CSF/side, downward triangles, n = 5, sites plotted in Fig. 8B) failed to prevent suppression of the JOR by i.t. DAMGO.

Figure 6

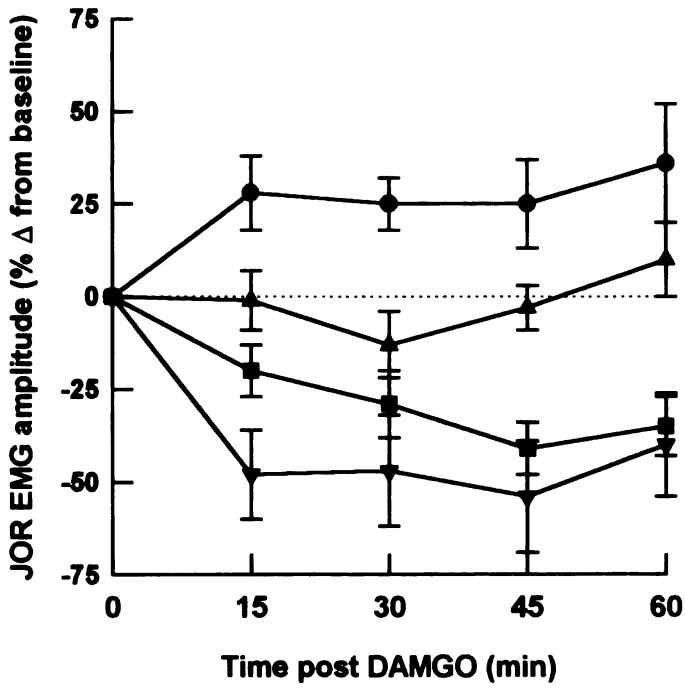

75
50
25
0
-25
-50
-75
JOR EMG amplitude (% Δ from baseline)
0
15
30
45
60
Time post DAMGO (min)

Figure 7.

The effect of i.t. lidocaine on the JOR with naloxone methiodide microinjected into specific basal forebrain sites. Naloxone methiodide (1 μg in 0.2 μl CSF/side, circles, n = 6, sites plotted in Figure 9A) microinjected into nucleus accumbens five minutes before i.t. lidocaine prevented suppression of the JOR. Offsite injections of naloxone methiodide (1 μg in 0.2 μl CSF/side, squares, n = 5, sites plotted in Figure 9B) failed to prevent suppression of the JOR by i.t. lidocaine.

Figure 7

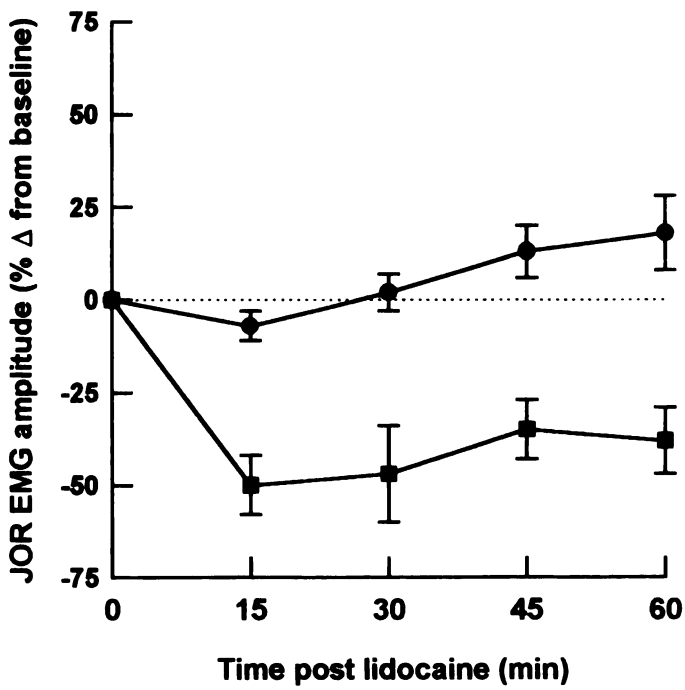

75
50
25
0
-25
-50
-75
JOR EMG amplitude (% Δ from baseline)
0
15
30
45
60
Time post lidocaine (min)

Figure 8.

A. Locations of naloxone methiodide injections plotted on coronal sections of PAG. In this and following figures numbers refer to distance (mm) caudal (negative numbers) or rostral to the interaural line of coronal sections adapted from the atlas of Paxinos and Watson (Paxinos and Watson 1986)

B. Locations of naloxone methiodide plotted on coronal sections of RVM adapted from the atlas of Paxinos and Watson (Paxinos and Watson 1986). VII, facial nucleus; P, pyramidal tract.

Figure 8

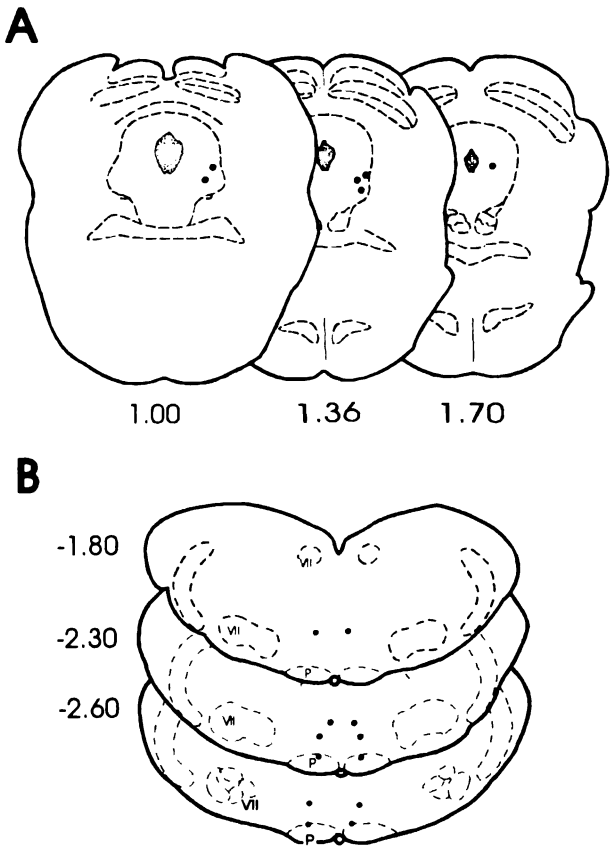
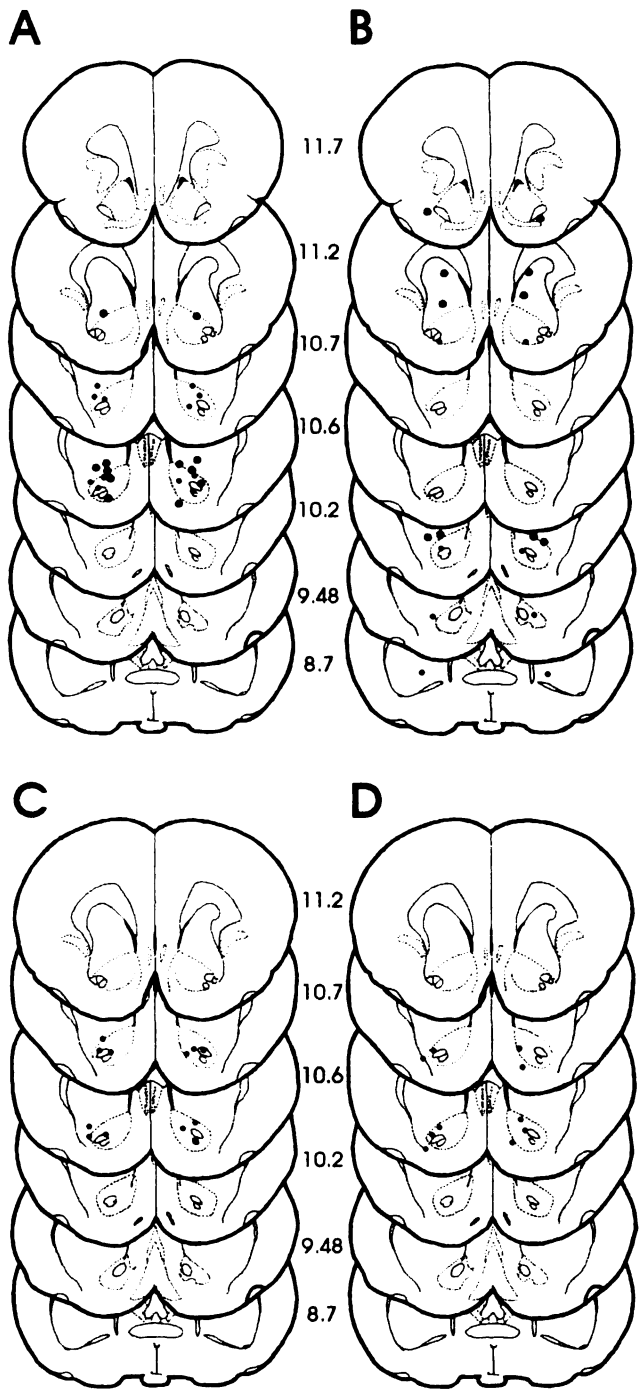


Figure 9.

Locations of injection sites in nucleus accumbens. Closed circles (except Panel D) indicate that the spinal drug was DAMGO, open circles indicate that the spinal drug was lidocaine. Panel A, "onsite" (i.e. in nucleus accumbens) locations of naloxone methiodide injections; Panel B, "offsite" (i.e., adjacent to nucleus accumbens) locations of naloxone methiodide injections; Panel C, "onsite" locations of CSF injections; and Panel D, "onsite" locations of naloxone methiodide injected as a single agent.

Figure 9



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Chapter 2

Nucleus Accumbens Mechanisms Mediating Ascending Nociceptive Control

Abstract

In addition to exerting a local inhibitory effect on nociceptive reflexes, spinal opioids induce antinociception via an ascending nociceptive control that is dependent on an opioidergic link in the nucleus accumbens (NAC) (Gear and Levine 1995). In this study, we identify the opioid receptor types in NAC that comprise this opioidergic link and examine the downstream effects of activation of these receptors.

To determine the opioid receptor type(s) in NAC mediating the ascending control, we tested the ability of receptor-selective opioid antagonists injected into NAC to prevent the attenuation of the JOR produced by spinal intrathecal (i.t.) [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO), a μ-opioid. We also tested the ability of receptor selective opioid agonists administered into NAC to attenuate the nociceptive jaw-opening reflex (JOR). While injection of either Cys^2,Tyr^3,Orn^5,Pen^7 amide (CTOP) or naltrindole (μ– and δ–antagonists, respectively) prevented attenuation of the JOR by i.t. DAMGO, neither DAMGO nor D-Pen^{2,5}-enkephalin (DPDPE), a δ-opioid, injected singly into NAC, even in high dose, affected the JOR. However, administration of a combination of low doses of DAMGO plus DPDPE attenuated the JOR.

To test the hypothesis that the μ-/δ-opioid combination in NAC produces antinociception by inhibiting efferent activity from NAC, we tested the ability of injection of lidocaine into NAC to inhibit the JOR. Intra-accumbens lidocaine

attenuated the JOR by an amount similar to that produced by injection of the μ-/δ-opioid combination into NAC. To test the hypothesis that NAC lidocaine and NAC μ-/δ-opioid combination produce antinociception by the same mechanism, we determined the ability of the μ-/δ-opioid combination to occlude production of antinociception by NAC lidocaine. Lidocaine injected into NAC 30 min after DAMGO plus DPDPE did not further attenuate the JOR.

Injection of the $GABA_A$ receptor agonist muscimol, but not naloxone, into the rostral ventral medulla (RVM) blocks the ascending antinociceptive effects of spinal opioids (Gear and Levine 1995). RVM muscimol, but not naloxone, also blocked the antinociceptive effect of μ-/δ-opioid combination or lidocaine injected into the NAC. In contrast, injection of naloxone as well as muscimol into the RVM blocked attenuation of the JOR that results from activation of the descending nociceptive control after injection of DAMGO into the periaqueductal gray (PAG).

Taken together, these results suggest that the ascending nociceptive control produces behavioral antinociception by inhibiting efferent activity from NAC, that this efferent inhibition requires co-activation of μ– and δ–opioid receptors in NAC, and that RVM GABAergic circuitry can exert a downstream effect on the ascending nociceptive control. It is possible that NAC efferent activity facilitates RVM GABAergic activity, and inhibition of this NAC efferent activity produces antinociception by removing (i.e., de-facilitating) RVM GABAergic activity.

Introduction

Although opioid receptors are well-known to be associated with the modulation of nociception and have been reported to be present in the nucleus accumbens (NAC) (Mansour, Khachaturian et al. 1987; Mansour, Thompson et al. 1993; George, Zastawny et al. 1994; Mansour, Fox et al. 1994; Minami, Onogi et al. 1994; Zastawny, George et al. 1994; Mansour, Fox et al. 1995), most studies involving NAC opioid receptors have focused on their role in the "reward pathway" extending from the ventral tegmental area to NAC, in opioid tolerance and dependence (for review see Di Chiara and North 1992), and on their roles in effecting changes in locomotor activity and in mediating learning in reward/aversive stimulus paradigms (for review see Pennartz, Groenewegen et al. 1994). A number of studies have reported that intra-accumbens morphine is antinociceptive (Dill and Costa 1977; Xuan, Shi et al. 1986; Yu and Han 1989; Yu and Han 1990; Yu and Han 1990; Tseng and Wang 1992), and that intra-accumbens naloxone attenuates the antinociceptive effect of systemically administered morphine (Dill and Costa 1977; Daghero, Bradley et al. 1987), but the role of NAC opioidergic circuitry in analgesic mechanisms has not been extensively investigated.

We have recently provided evidence that opioid circuitry in NAC plays an important role in a novel ascending nociceptive control (Gear and Levine 1995). Spinal administration of the μ-opioid [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) attenuates the trigeminal nociceptive jaw-opening reflex (JOR), and this attenuation is blocked by prior administration of the non-selective opioid antagonist naloxone to the NAC. In the present experiments we have studied the NAC opioidergic circuitry mediating the ascending nociceptive control. We also examined the effect of activation of NAC opioid receptors on NAC efferent

activity. Finally, data is presented to support the suggestion that the mechanism of antinociception produced by activation NAC opioidergic circuitry and the ascending control differs from the mechanism of antinociception produced by activation of the descending nociceptive control system.

Some of these results have been previously reported in abstract form (Gear and Levine 1995).

Materials and Methods

The experiments were performed on 250 - 450 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) that were lightly anesthetized by intraperitoneal injection of 0.9 gm/kg urethane and 45 mg/kg α-chloralose (both from Sigma, St. Louis, MO), and 10 mg/kg methohexital (Brevital, Eli Lilly, Indianapolis, IN) for rapid induction of anesthesia. The urethane/α-chloralose combination was chosen for anesthesia because this anesthetic provides a stable JOR EMG over the time period required to complete the experiments (Gear and Levine 1995).

Changes in nociception were measured as changes in jaw-opening reflex (JOR) electromyographic (EMG) amplitude (Gear and Levine 1995). A bipolar stimulating electrode, fabricated from two insulated single-stranded 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 (Toda, Iriki et al. 1981). Access to the pulp of the incisor was through an opening in the labial surface of the tooth starting 2 mm below the gingival crest and extending 4 mm toward the incisal edge. Dental composite resin was used to cement the electrode in place and seal the opening in the tooth. 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 digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire. A 22-gauge needle inserted in the skin ventral to the midline of the mandible was used as a ground connection. The JOR was activated by stimulating the tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Stimulation voltage was adjusted to evoke

maximum electromyographic (EMG) signal (approximately 3 times the threshold voltage).

Spinal intrathecal (i.t.) administration of drugs was through a polyethylene catheter 10 μl in volume (Intramedic PE-10 tubing, VWR Scientific, San Francisco, CA) inserted 8.5 cm caudally into the subarachnoid space through a slit in the atlanto-occipital membrane (Yaksh and Rudy 1976). For supraspinal sites 25 gauge guide cannulae (made from hypodermic needles, Smith & Nephew MPL, Franklin Park, IL) were stereotactically positioned and cemented with orthodontic resin (L.D. Caulk Co., Milford, DE) to allow injections via insertion of a 33 gauge injection cannula (stainless steel tubing, Small Parts, Inc., Miami Lakes, FL) connected to a 2 μl syringe (Hamilton, Reno, NV). PAG injection cannulae were angled 12° to the right of vertical; all others were vertically positioned. Injection sites were verified by histological examination (70 μm sections stained with cresyl violet acetate) and were plotted on coronal sections adapted from the atlas of Paxinos and Watson 1986.

Twelve consecutive evoked JOR EMG signals were averaged per recording. Effects of experimental interventions on the JOR EMG were measured as the percentage change from the average amplitude of three baseline recordings taken at five minute intervals. Data is expressed as the mean percentage change ± s.e.m. for each experimental group. Repeated measures ANOVA with Fisher's post hoc test (Fisher 1949) or the Student-Neuman-Keuls test were used as appropriate to compare groups for significant differences ($p < 0.05$).

Drugs. [D-Ala^2, N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO), D-Pen^{2,5} enkephalin (DPDPE) (Sigma, St. Louis, MO), Cys^2,Tyr^3,Orn^5,Pen^7 Amide (CTOP) (Peninsula Laboratories, Belmont, CA), naloxone methiodide, and muscimol hydrobromide (Research Biochemicals International, Natick, MA) were dissolved

in artificial CSF. Naltrindole and lidocaine N-ethyl bromide salt (QX-314) (Research Biochemicals International, Natick, MA) were dissolved in distilled water. QX-314 and naloxone methiodide are quaternary derivatives of lidocaine and naloxone, respectively, used to retard drug spread from the site of injection (Schroeder, Weinger et al. 1991). Artificial CSF was made according to the following formula: NaCl (125.8 mM), $NaHCO_3$ (27.5 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $CaCl_2$ (1.1 mM), $MgCl_2$ (0.83 mM), Na_2SO_4 (0.5 mM), glucose (5.0 mM); pH adjusted to 7.3-7.4, osmolality adjusted to 310 with sucrose (Leviel, Gobert et al. 1989). To retard rostral flow of the drugs administered i.t., all animals were placed in a prone position on an inclined surface (approximately 30 degrees) with the head higher than the tail. I.t. drug or vehicle volumes were 15 µl followed by 10 µl of vehicle (equal to the volume of the i.t. catheter). Injections into specific supraspinal sites were carried out over a period of 90 seconds, and the injection cannulae were left in place an additional 30 seconds after injection.

Results

Receptor selective opioids and local anesthetic in nucleus accumbens

Receptor selective opioid antagonists

To determine whether μ- or δ-opioid receptors in NAC mediate the ascending control, i.t. DAMGO was administered 5 minutes after injection into NAC of either the μ-receptor selective antagonist CTOP or the δ-receptor selective antagonist naltrindole or their vehicles. DAMGO was also administered i.t. as a single agent as a control. Attenuation of the JOR by i.t. DAMGO was blocked by either CTOP or naltrindole, but not by their vehicles ($p < 0.05$ in both cases), indicating that the NAC opioid link mediating the ascending control involves both μ- and δ-opioid receptors. Also, although there is a trend toward greater maximal attenuation of the JOR in the presence of either vehicle than with i.t. DAMGO alone, this difference is not statistically significant (Fig. 1).

Receptor selective opioid agonists

To determine if either a μ- or δ-selective opioid agonist administered into NAC attenuates the JOR, either DAMGO or DPDPE (selective agonists for μ- or δ-receptors, respectively) was bilaterally injected into NAC. Neither of these agents significantly affected the amplitude of the JOR EMG (Fig. 2). However, since either a μ- or δ- antagonist blocked attenuation of the JOR by i.t. DAMGO, we investigated the possibility that co-activation of NAC μ- and δ-receptors might be necessary to attenuate the JOR. Therefore, a combination of DAMGO plus DPDPE was bilaterally injected into NAC in three increasing concentrations (cumulative doses). Intra-accumbens injection of this opioid combination attenuated the JOR in a dose-related fashion (Fig. 2), but extra-accumbens injection (i.e., offsite) did not significantly affect the JOR. These results suggest

that the NAC-opioid link mediating the ascending control is dependent on co-activation of both μ- and δ-opioid receptors.

NAC lidocaine

To determine if attenuation of NAC efferent activity attenuates the JOR, lidocaine (QX-314) was injected as a single agent into the NAC (Fig. 3). QX-314 (same dose) injected into sites surrounding NAC (i.e., offsite injections) did not significantly affect the JOR. To determine if the mechanisms by which either lidocaine or DPDPE plus DAMGO attenuate the JOR are similar, an "occlusion" experiment was performed. Injection of DPDPE plus DAMGO into NAC was followed, thirty minutes later, by injection of QX-314 into the same site. QX-314 did not significantly affect attenuation of the JOR produced by the opioid combination (Fig. 4) suggesting: 1) that prior administration of the opioid combination occluded the ability of lidocaine to further attenuate the JOR, and 2) that opioids produce antinociception by inhibition of NAC efferent activity.

NAC - RVM interactions.

RVM GABAergic circuitry is downstream of NAC.

We have reported, in abstract form, that antinociception produced by either the ascending or the descending control can be modulated by RVM GABAergic circuitry (Gear and Levine 1995). This conclusion was based on our observations that attenuation of the JOR is blocked by intra-RVM injection of the $GABA_A$ agonist muscimol 10 min prior to administration of DAMGO either spinally (i.t.) (Fig. 5) or into PAG (Fig. 6). To determine if NAC-induced antinociception is similarly mediated by RVM GABAergic circuitry, the μ-/δ-opioid combination or QX-314 was bilaterally microinjected into NAC with or without prior (10 min) injection of muscimol into the RVM. RVM muscimol blocked attenuation of the JOR by both of these NAC treatments (Fig. 7). These findings are consistent with

the suggestion that an RVM GABAergic link mediates ascending control and that this RVM link is downstream from NAC in the ascending control circuit.

RVM opioidergic circuitry is not downstream of NAC.

RVM opioidergic circuitry does not appear to be involved in the ascending control (Gear and Levine 1995). To determine if RVM opioidergic circuitry mediates the NAC-induced antinociception, DPDPE plus DAMGO or QX-314 was bilaterally microinjected into NAC 10 min after bilateral injection of naloxone methiodide into the RVM (Fig. 8). Consistent with the finding involving spinal DAMGO, intra-RVM naloxone failed to prevent attenuation of the JOR by these NAC treatments suggesting that RVM opioidergic circuitry does not mediate NAC-induced or spinally-induced antinociception. In contrast, naloxone methiodide blocked antinociception induced by intra-PAG injection of DAMGO (Fig. 9) confirming in the JOR model that the descending control system is mediated by an opioidergic link in the RVM.

Discussion

The major findings of this study and the previous study (Gear and Levine 1995) are depicted in figure 10.

NAC opioid receptors

In this study we show that the NAC opioid link that mediates antinociception by the ascending nociceptive control depends on co-activation of μ- and δ-opioid receptors. This conclusion is based on the following findings: 1) attenuation of the JOR by i.t. DAMGO is blocked by intra-accumbens injection of either the selective μ-antagonist CTOP or the selective δ-receptor antagonist naltrindole; 2) high dose of the selective μ-agonist DAMGO or the selective δ-agonist DPDPE, injected into NAC as a single agent, does not produce significant antinociception; and 3) intra-accumbens injection of a combination of DAMGO plus DPDPE, in smaller doses, induced a significant antinociceptive effect that increased with subsequently administered higher doses.

Action of opioids on NAC efferent activity

To test the hypothesis that the μ-/δ-opioid combination in NAC produces antinociception by inhibiting efferent activity from NAC, we tested the ability of injection of lidocaine into NAC to inhibit the JOR. Onsite, but not offsite, injections of lidocaine attenuated the JOR suggesting that inhibition of efferent activity from the NAC produces antinociception. To test the hypothesis that NAC lidocaine and NAC μ-/δ-opioid combination produce antinociception by the same mechanism, we determined the ability of the μ-/δ-opioid combination to occlude production of antinociception by NAC lidocaine. Lidocaine injected into NAC 30 min after DAMGO plus DPDPE did not further attenuate the JOR. This observation, plus the observation that intra-accumbens lidocaine, injected as a single agent, similarly attenuates the JOR, suggests that NAC-mediated

antinociception results from suppression of NAC efferent activity. This suggestion is compatible with the observations that 1) NAC efferent activity is mediated exclusively by spiny neurons that require excitatory synaptic input to be active (Chang and Kitai 1985; Pennartz, Boeijinga et al. 1991; Pennartz and Kitai 1991; Arts and Groenewegen 1992; but also see review in Pennartz, Groenewegen et al. 1994), and 2) that opioids reduce synaptic activity in an *in vitro* NAC slice preparation (Yuan, Madamba et al. 1992). Taken together, these results imply that, since attenuation of NAC efferent activity produces antinociception, unattenuated NAC efferent activity facilitates nociception.

RVM circuitry mediating the ascending nociceptive control

Our findings that injection into the RVM of the $GABA_A$-receptor agonist muscimol prevents attenuation of the JOR by either i.t. DAMGO, intra-accumbens opioid combination, or intra-accumbens lidocaine implicate RVM GABAergic circuitry in the mediation of the ascending control regardless of the point in the circuit of drug application (i.e., the spinal cord or the NAC) thus suggesting that the RVM is downstream of the NAC in the ascending control. Although direct neuronal projections from NAC to RVM have not, to our knowledge, been reported, NAC has been described to project to other brainstem sites, for example, the PAG (Groenewegen and Russchen 1984) that, in turn, project to the RVM (see Pennartz, Groenewegen et al. 1994 for review of the efferent connections of NAC). Indirect evidence that NAC efferent activity reaches the RVM was recently reported in a study that showed that RVM neurons are excited by electrical stimulation of either the NAC or somatosensory area II, and lesion of NAC blocked the ability of Sm II stimulation to modulate RVM neuronal activity (Jiang and Liu 1993).

It has been proposed that antinociceptive efferent signals from the RVM are under tonic GABAergic inhibition (Cho and Basbaum 1991; Fields, Heinricher et al. 1991; Heinricher, Haws et al. 1991; Heinricher and Kaplan 1991). The ability of a $GABA_A$-receptor agonist (muscimol) to antagonize the antinociceptive effect of the ascending control suggests that the ascending control produces antinociception by reducing RVM GABAergic activity. One manner in which GABAergic activity might be reduced is by post-synaptic inhibition of GABAergic neurons, which has been proposed as a mechanism by which opioids act to produce antinociception. However, since I have demonstrated that intra-RVM naloxone does not block activation of the ascending control, any RVM post-synaptic inhibition mediated by the ascending control would likely be non-opioidergic. Another mechanism by which RVM GABAergic activity could be reduced by activation of the ascending control is through pre-synaptic inhibition. Pre-synaptic inhibition could take the form of reduced excitatory afferent drive to RVM GABAergic neurons (i.e., de-facilitation of RVM GABAergic neurons). Evidence compatible with the pre-synaptic inhibition hypothesis has been reported in studies of RVM "on-cell" and "off-cell" response to morphine administered either intrathecally or into PAG, or lidocaine administered intrathecally (Heinricher and Drasner 1991; Morgan, Heinricher et al. 1992). Thus, the ascending control may produce antinociception by de-facilitation, as opposed to post-synaptic inhibition, of RVM GABAergic activity. Importantly, this suggestion is compatible with the evidence, discussed above, that NAC opioids produce antinociception by attenuating pro-nociceptive efferent activity from the NAC.

RVM circuitry mediating the descending nociceptive control

Whereas the antinociceptive effect of the ascending control may result from decreased afferent drive to RVM GABAergic circuits, the descending control has been proposed to act by a post-synaptic inhibitory mechanism in the RVM (Morgan, Heinricher et al. 1992). To compare these two systems using the JOR model, we activated the descending nociceptive control by injecting DAMGO into the PAG and determining whether either opioidergic or GABAergic circuitry in the RVM mediates the resulting antinociceptive effect. We demonstrate that attenuation of the JOR by DAMGO administered into the PAG is prevented by prior injection of either naloxone methiodide or muscimol into the RVM. These findings confirm the findings of others that the descending control is mediated by opioidergic (Kiefel, Rossi et al. 1993) as well as GABAergic circuitry (Cho and Basbaum 1991) in the RVM. Furthermore, these results are in contrast to our finding that the ascending control of the JOR is not mediated by RVM opioidergic circuitry. Thus, we suggest that the descending control is mediated, at least in part, by an opioidergic post-synaptic inhibitory mechanism in the RVM, and the ascending control is mediated by a pre-synaptic (de-facilitatory) mechanism in the RVM.

This difference provides support for the suggestion that the ascending and descending nociceptive controls may play opposing roles in modulating nociception. Thus, the ascending nociceptive control system, instead of being "antinociceptive," may function as a system that normally increases sensitivity to nociceptive stimuli but that is shut down by events that evoke the release of opioids in the spinal cord and/or in NAC. In contrast, under normal physiological conditions in which the descending control is presumed not to be activated, it has been proposed that the efferent antinociceptive activity from PAG is normally

under tonic GABAergic inhibition and that activation of the descending control disinhibits this antinociceptive signal. Finally, the observations that RVM GABAergic circuitry is implicated in both the ascending and the descending controls suggests that the RVM may serve to integrate these two systems.

Although this description of the ascending nociceptive control might also apply to the diffuse noxious inhibitory controls (DNIC), the ascending control appears to be mediated by different circuits than is DNIC. The antinociceptive effect of DNIC is mediated by ascending excitatory activity (Le Bars and Villanueva 1988; Villanueva, Bouhassira et al. 1988) whereas the ascending nociceptive control produces activity when ascending spinal activity is inhibited (Gear and Levine 1995); also, DNIC is not mediated in the RVM (Bouhassira, Chitour et al. 1993) in contrast to the ascending antinociceptive control which is mediated by an RVM GABAergic link.

In summary, we provide evidence that the NAC circuitry mediating the ascending nociceptive control involves μ- and δ-opioidergic synapses, that efferent activity from NAC may facilitate pro-nociceptive RVM GABAergic activity, and that antinociception induced by the ascending and descending controls is distinguishable on the basis of involvement of RVM opioids.

Figures

Figure 1.

A. The effect of selective opioid antagonists injected into the NAC on attenuation of the JOR by i.t. DAMGO. DAMGO (7.5 μg in 15 μl saline) was administered i.t. as a single agent (◆), n = 17, or 5 minutes after injection of either CTOP (●), 1 μg in 0.3 μl CSF per side, n = 5; naltrindole (■), 1 μg in 0.3 μl water per side, n = 5; vehicle for CTOP (○), 0.3 μl CSF per side, n = 7; or vehicle for naltrindole (□), 7.5 mM saline--same concentration as naltrindole--0.3 μl per side, n = 4. Two-way repeated measures ANOVA with one between subjects factor (treatment) and one within subjects factor (time) revealed significant difference between the groups. Fisher's posthoc analysis showed that both groups that received antagonist were significantly different from the other groups, but not significantly different from each other. Although the groups that received vehicle into NAC tended to show greater JOR attenuation than the group given DAMGO i.t. as a single agent, these three groups are not statistically different from each other. In this and subsequent figures data points are plotted as mean ± s.e.m.

B. NAC injections sites for CTOP (●), CTOP vehicle (○), naltrindole (■), and naltrindole vehicle (□). In this and following figures numbers refer to distance (mm) caudal (negative numbers) or rostral to the interaural line (Paxinos and Watson 1986).

Figure 1A

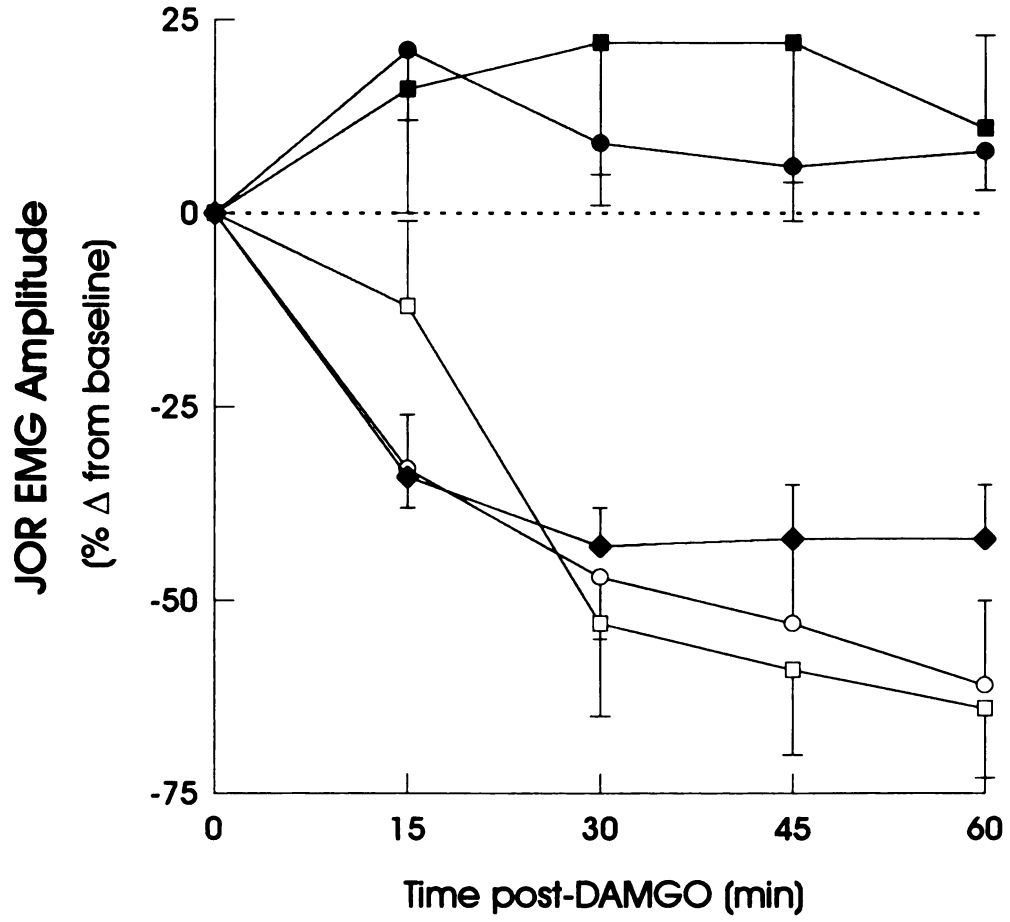

JOR EMG Amplitude
(% Δ from baseline)
25
0
-25
-50
-75
0
15
30
45
60
Time post-DAMGO (min)

Figure 1B

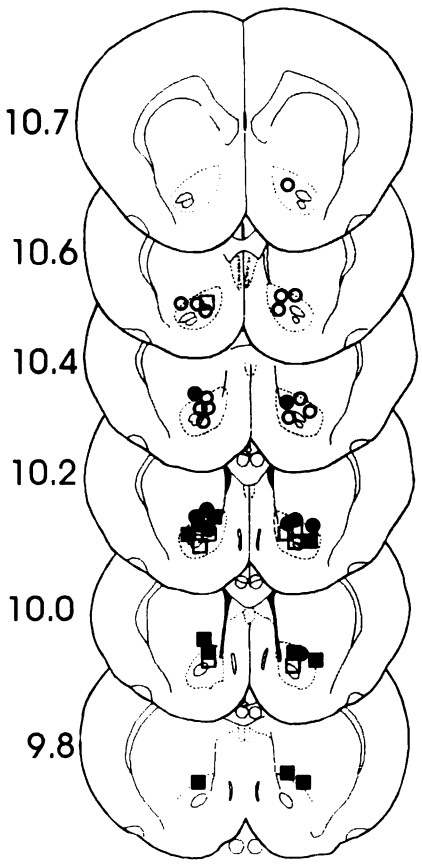


Figure 2.

A. The effect on the JOR of selective opioid agonist(s) injected into the NAC. DAMGO, 150 ng in 0.3 μl CSF per side, n = 6, or DPDPE, 150 ng in 0.3 μl CSF per side, n = 6, failed to significantly attenuate the JOR 30 min after injection into NAC. However, the combination of DAMGO plus DPDPE, 15, 45 and 150 ng of each opioid in 0.3 μl CSF per side, administered cumulatively, n = 8, attenuated the JOR in a dose-related fashion. The JOR baseline, recorded before the first dose, was used as baseline for all three doses; JOR was measured 30 minutes after administration of each dose (just prior to administration of the next dose). Offsite injections: bilateral administration of DAMGO plus DPDPE, 150 ng of each opioid in 0.3 μl CSF per side, n = 3, into sites adjacent to the NAC. ANOVA demonstrated significant difference between the groups; posthoc Student-Neuman-Keuls analysis showed that DAMGO alone, DPDPE alone, low dose combination, and Offsite groups were not significantly different from each other. However, the middle and high doses of the opioid combination were significantly different from the other groups.

B. NAC injection sites for opioid agonists. DAMGO 150 ng (△), DPDPE 150 ng (▽), DAMGO plus DPDPE cumulative dose group (■), offsite injections (□).

Figure 2A

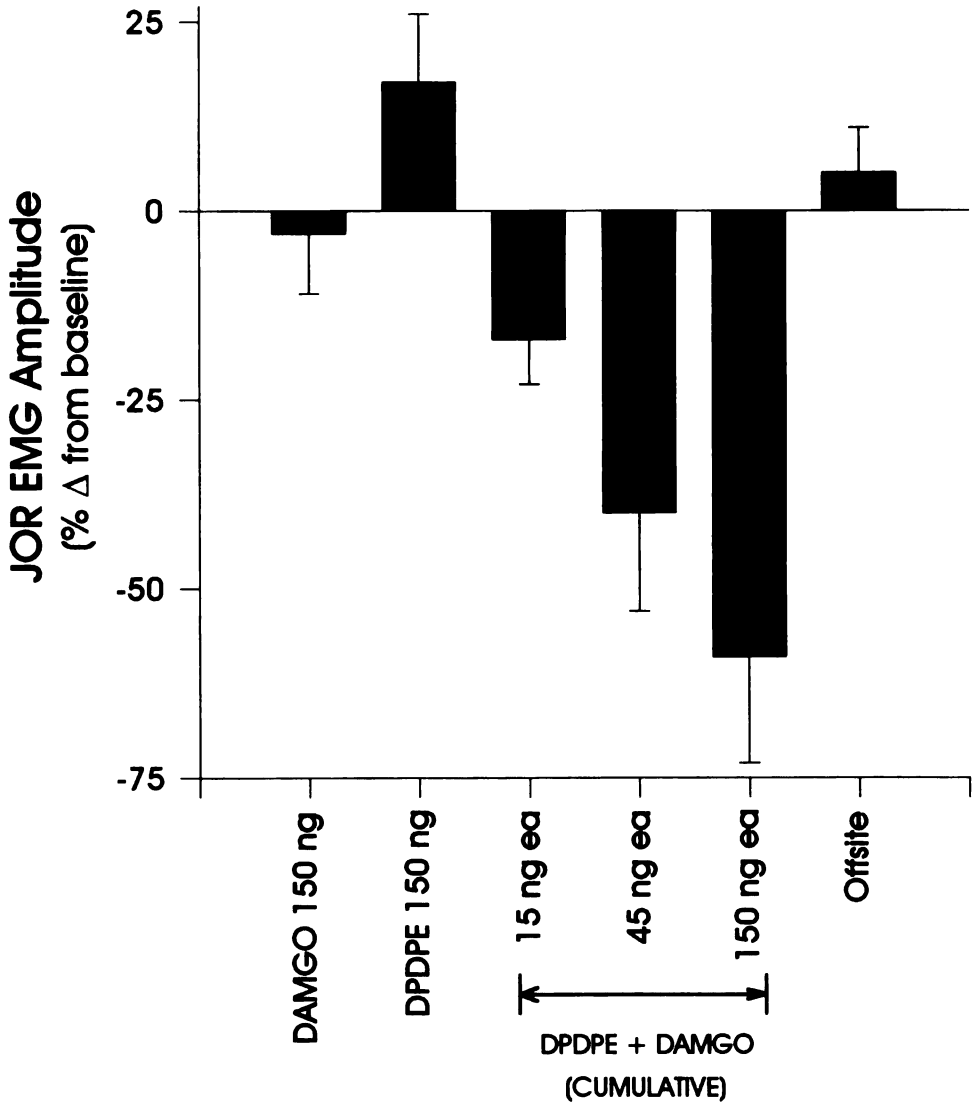

JOR EMG Amplitude
(% Δ from baseline)
25
0
-25
-50
-75
DAMGO 150 ng
DPDPE 150 ng
15 ng ea
45 ng ea
150 ng ea
Offsite
DPDPE + DAMGO
(CUMULATIVE)

Figure 2B

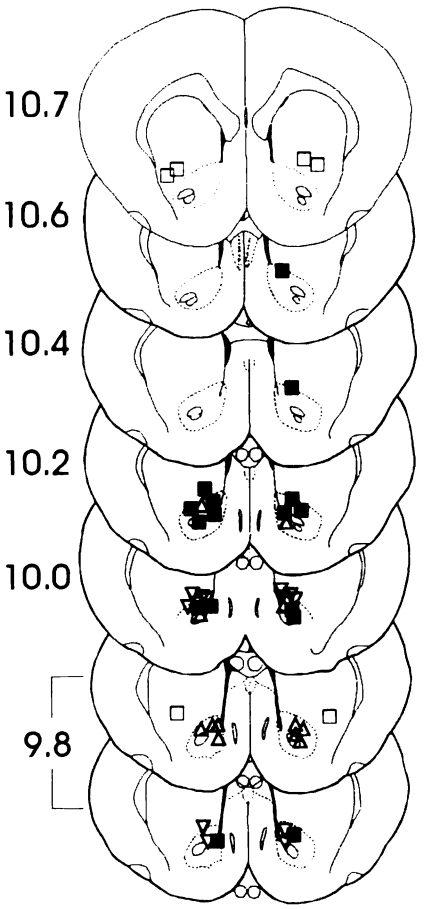


Figure 3.

A. The effect on the JOR of lidocaine injected into the NAC. Lidocaine (●), QX-314, 333 ng in 0.3 μl H_20 per side, n = 7, or offsite lidocaine (■), QX-314, same dose, n = 7, were injected in NAC. Repeated measures ANOVA with one between subjects factor (treatment/site) and one within subjects factor (time) demonstrated significant difference between the two groups.

B. NAC injection sites for animals receiving either lidocaine (●), offsite lidocaine (■).

Figure 3A

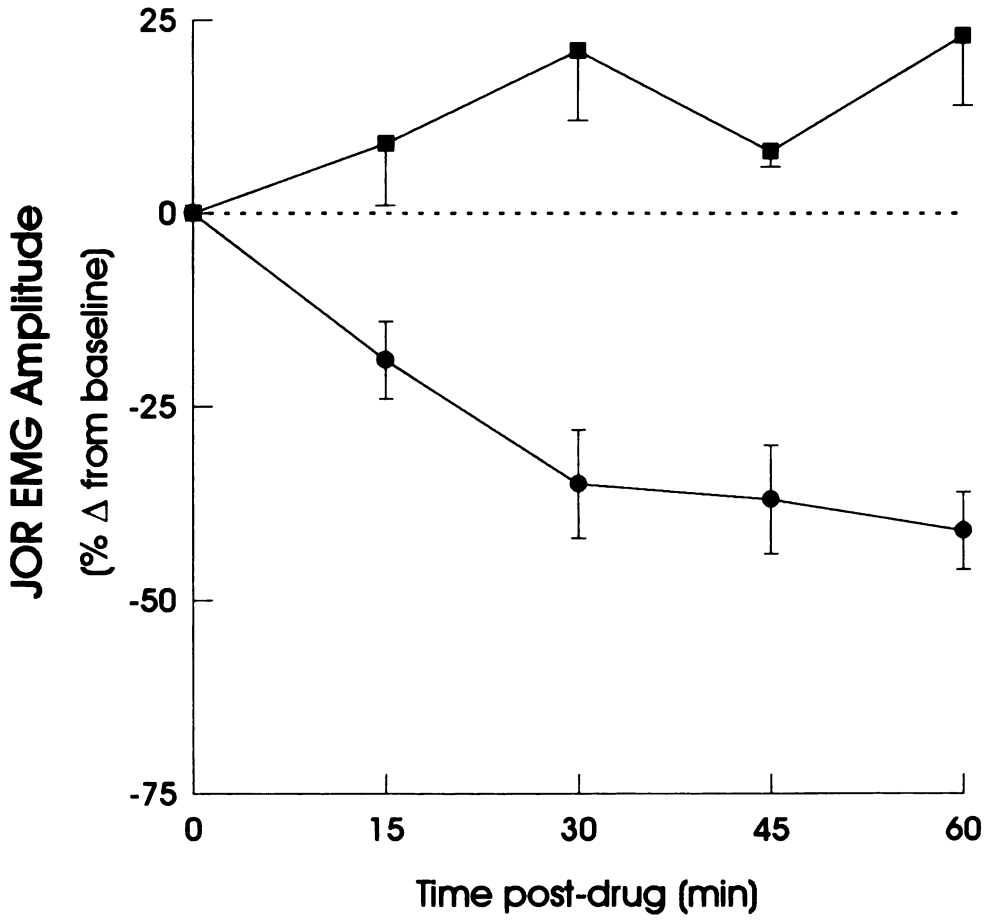

25
0
-25
-50
-75
JOR EMG Amplitude
(% Δ from baseline)
0
15
30
45
60
Time post-drug (min)

Figure 3B

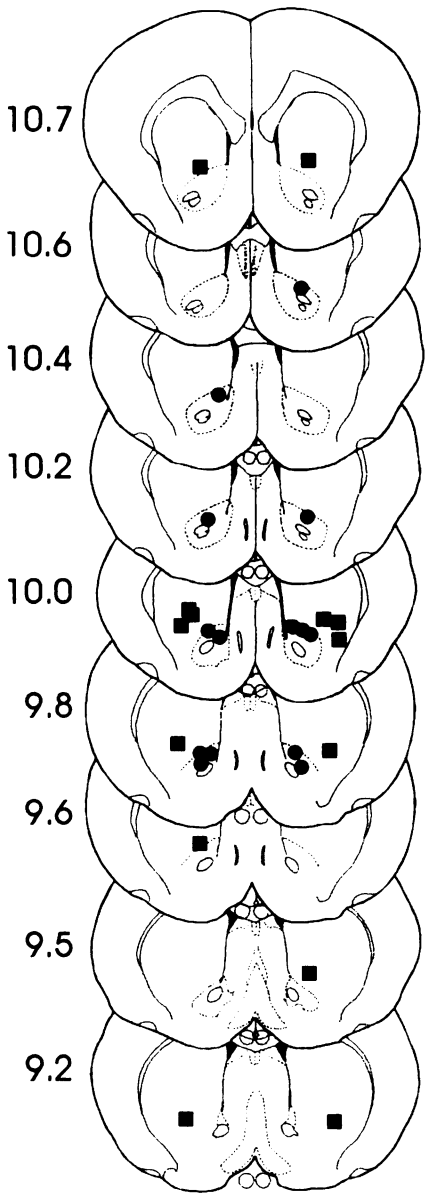


Figure 4.

A. The effect of lidocaine injected into the NAC on attenuation of the JOR produced by previous injection of DAMGO plus DPDPE into the NAC. Intra-accumbens injection of DAMGO plus DPDPE, 150 ng of each opioid in 0.3 µl CSF per side, n = 5, was followed by injection of lidocaine, QX-314, 333 ng in 0.3 µl H_20 per side into the same site. The JOR recorded prior to the first injection was used as baseline to calculate attenuation of the JOR 30 minutes after injection of the opioid combination (immediately prior to lidocaine administration) and also 30 minutes after the injection of lidocaine. Lidocaine did not significantly affect attenuation of the JOR produced by the opioid combination.

B. NAC injection sites for lidocaine and DAMGO plus DPDPE (◆).

Figure 4A

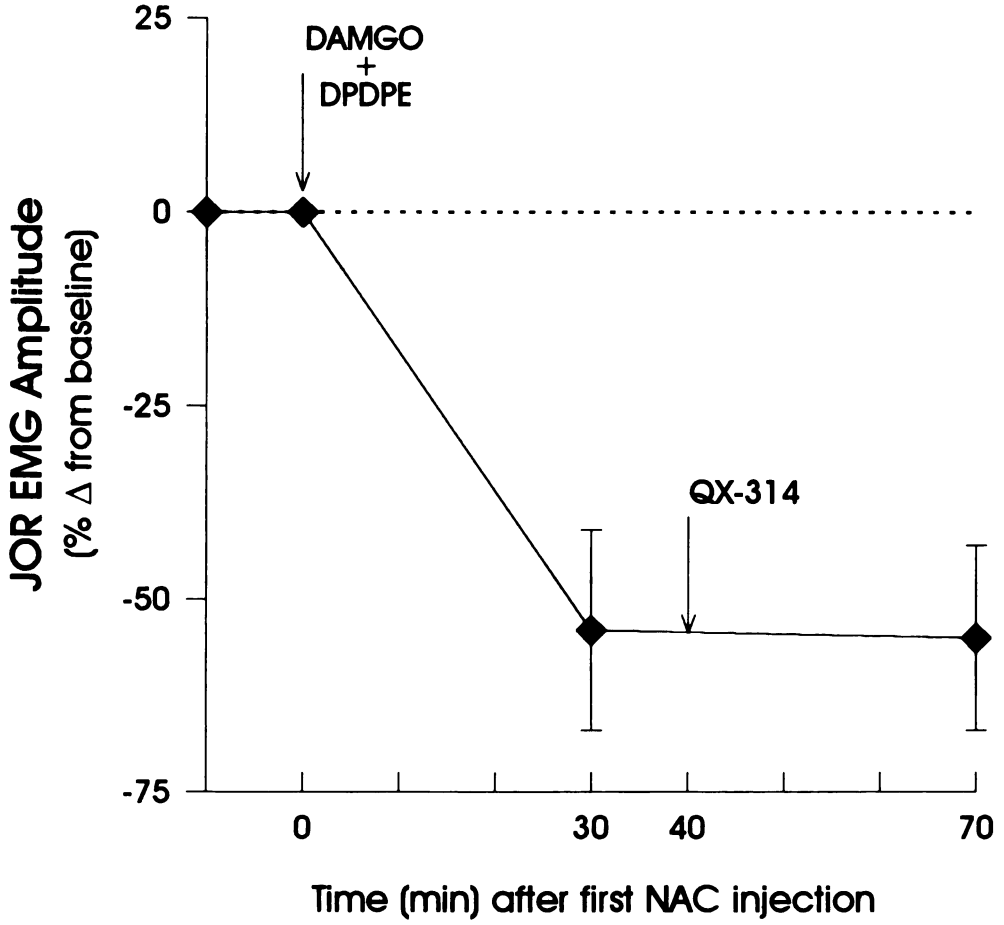

25
0
-25
-50
-75
DAMGO
+
DPDPE
QX-314
JOR EMG Amplitude
(% Δ from baseline)
0
30
40
70
Time (min) after first NAC injection

Figure 4B

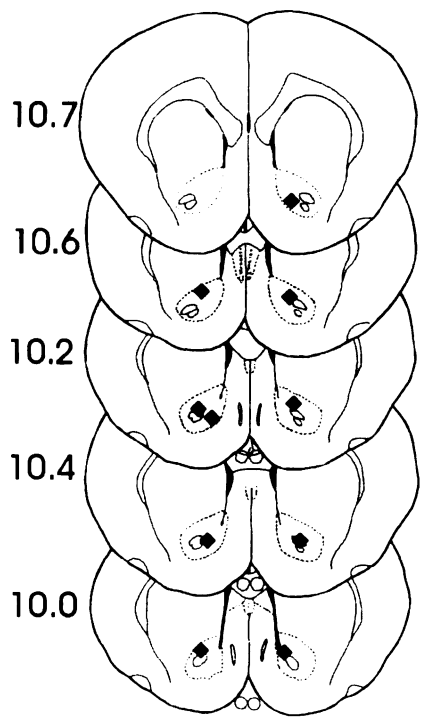


Figure 5.

A. Effect of muscimol injected into the RVM on attenuation of the JOR by i.t. DAMGO. DAMGO, 7.5 μg in 15 μl 0.9% saline, was administered i.t. 10 minutes after injection into the RVM of either vehicle (△), 0.5 μl 0.9% saline, n = 7, or the $GABA_A$ receptor selective agonist muscimol, 20 ng in 0.5 μl 0.9% saline, administered either onsite (▲), n = 6, or offsite (●), n = 6. Also, i.t. vehicle, 15 μl 0.9% saline, was administered 10 minutes after onsite injection of muscimol (○), same dose, n = 4. Repeated measures ANOVA with one between subjects factor (treatment/site) and one within subjects factor (time) demonstrated significant difference between the groups. Fisher's posthoc analysis showed that the group receiving onsite muscimol and i.t. DAMGO was not significantly different from the group receiving onsite muscimol and i.t. vehicle. Also the group receiving offsite muscimol and i.t. DAMGO was not significantly different from the group receiving onsite vehicle and i.t. DAMGO. However, the latter two groups were both significantly different from the former two groups.

B. RVM injection sites: i.t. DAMGO/onsite RVM muscimol (▲), i.t. DAMGO/onsite RVM vehicle (△), i.t. DAMGO/ offsite RVM muscimol (●), i.t. vehicle/onsite RVM muscimol (○).

Figure 5A

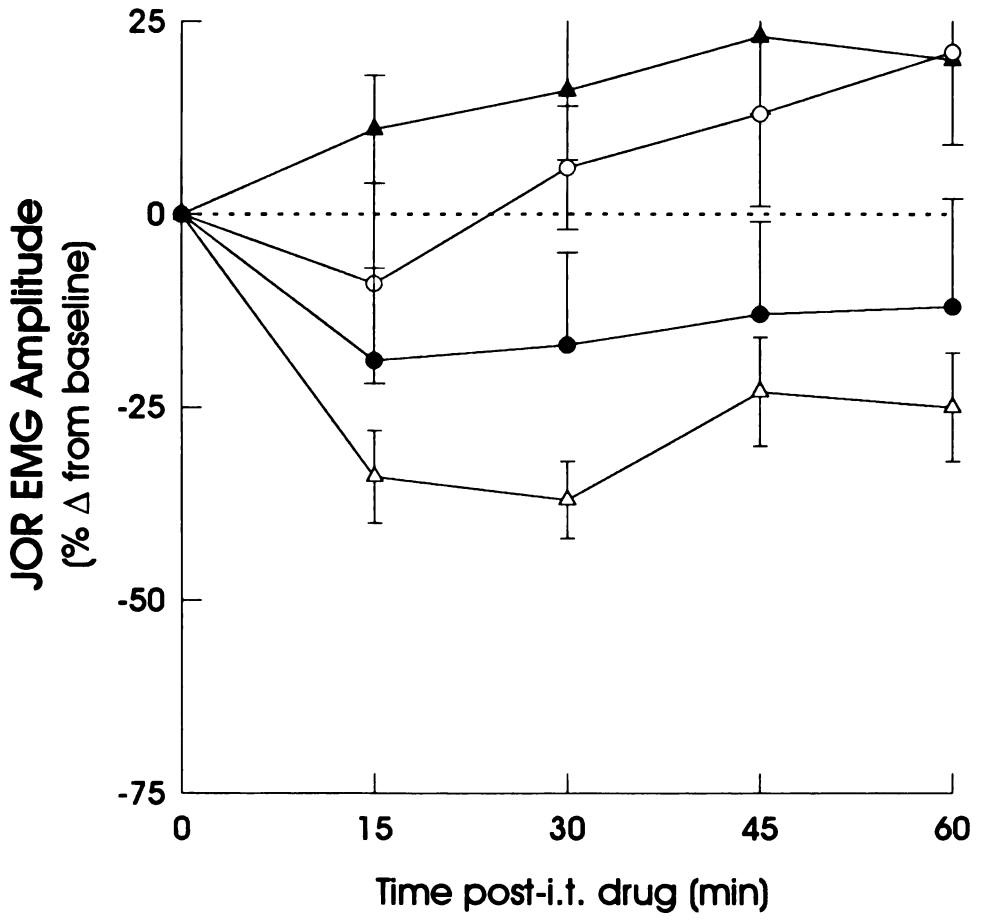

25
0
-25
-50
-75
JOR EMG Amplitude
(% Δ from baseline)
0
15
30
45
60
Time post-i.t. drug (min)

Figure 5B

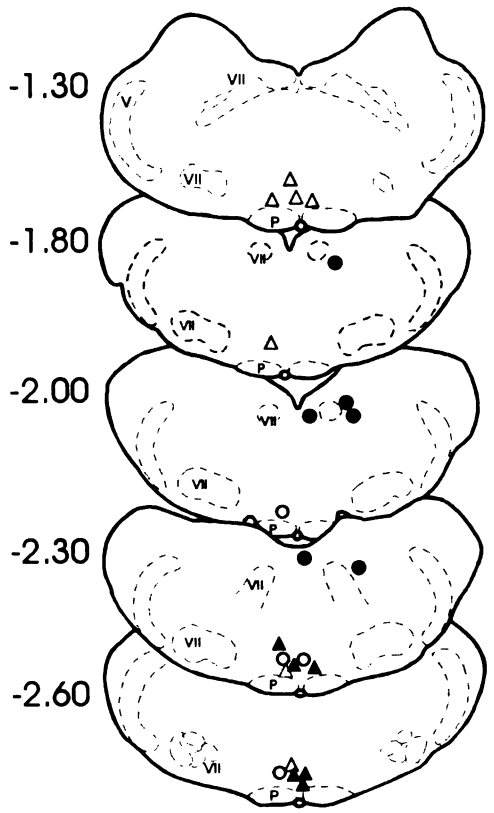


Figure 6.

A. The effect of muscimol injected into the RVM on attenuation of the JOR produced by DAMGO injected into the PAG. DAMGO, single injection, 12° from vertical, 60 ng in 0.3 μl CSF, was administered to the PAG 10 minutes after intra-RVM injection of the $GABA_A$ receptor selective agonist muscimol, 10 ng in 0.3 μl 0.9% saline either onsite (▲), n = 4, or offsite (O), n = 3. Data for injection of DAMGO into PAG following injection of vehicle into RVM are replotted from Fig. 9 (△). Repeated measures ANOVA with one between subjects factor (drug treatment/site) and one within subjects factor (time) demonstrated significant differences between the groups. Fisher's posthoc analysis showed that there was no significant difference between the groups receiving RVM vehicle or offsite muscimol. However, there was significant difference between these two groups and the group receiving onsite RVM muscimol.

B. PAG injection sites: animals receiving onsite muscimol (▲), or offsite muscimol (O).

C. RVM injection sites: animals receiving onsite muscimol (▲), or offsite muscimol (O).

Figure 6A

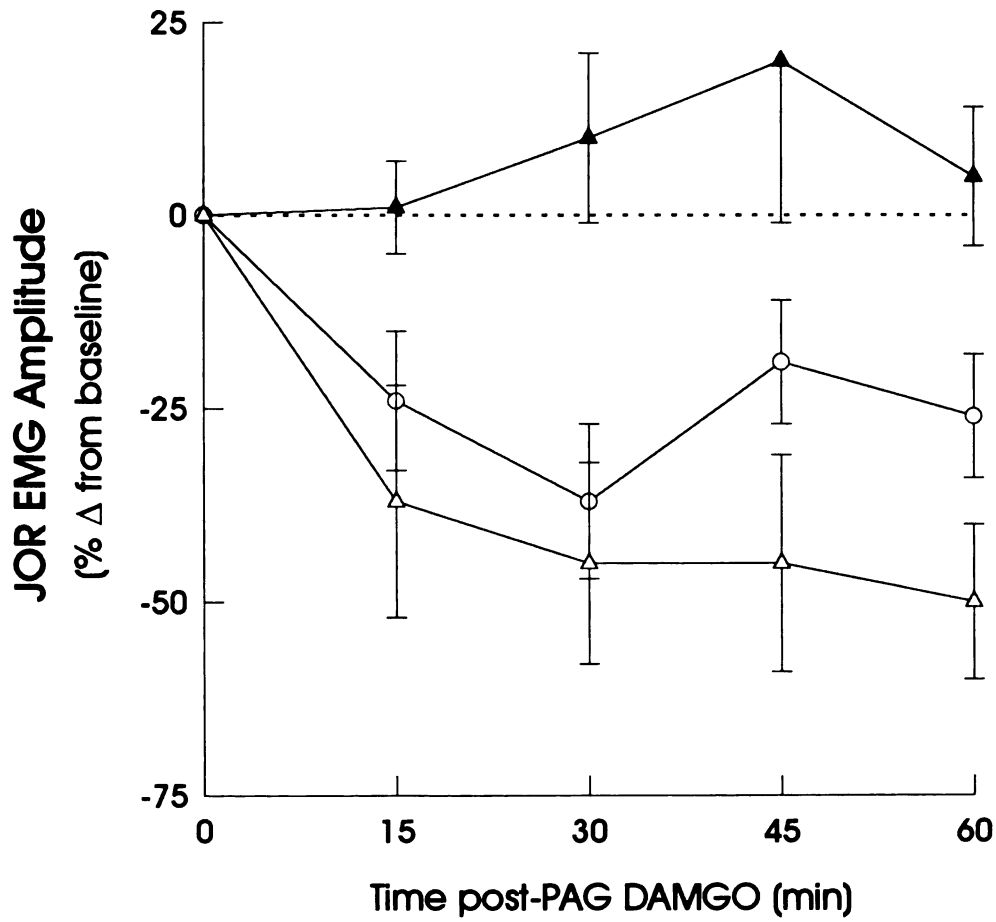
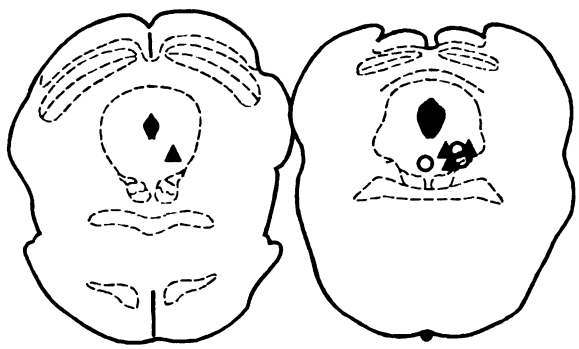

25
0
-25
-50
-75
0
15
30
45
60
JOR EMG Amplitude
(% Δ from baseline)
Time post-PAG DAMGO (min)

Figure 6B/C

B



1.70 1.00

C

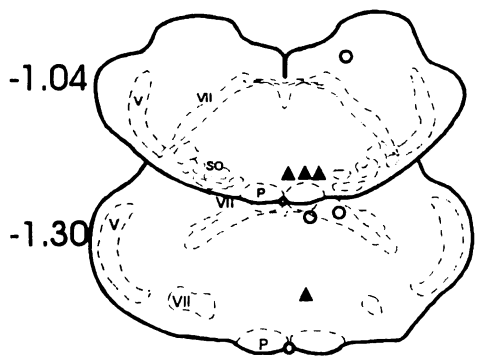


Figure 7.

A. The effect of injection of muscimol into the RVM on attenuation of the JOR produced by either DAMGO plus DPDPE or lidocaine injected into the NAC. Muscimol, 20 ng in 0.3 μl 0.9% saline, was injected into the RVM 10 minutes prior to administration of either DAMGO plus DPDPE, 150 ng of each opioid in 0.3 μl CSF per side, n = 4, or QX-314, 333 ng in 0.3 μl H_2O per side, n = 6, into the NAC. The JOR was recorded thirty minutes after injection of either lidocaine or the opioid combination into NAC. For comparison, the effect on the JOR, at the same time point, of these same NAC treatments in the absence of RVM muscimol are replotted from Fig. 2 (DAMGO plus DPDPE, same dose) or Fig. 3 (lidocaine, same dose). Muscimol blocked attenuation of the JOR by either of these NAC treatments. ANOVA demonstrated significant difference between the groups. Student-Neuman-Keuls posthoc analysis showed that the groups that received muscimol into the RVM were both significantly different from the other two groups, but were not significantly different from each other. Also, the groups that received the NAC treatments in the absence of intra-RVM muscimol were not statistically different from each other.

B. NAC injection sites for animals that received muscimol and either DAMGO plus DPDPE (□) or QX-314 (■).

C. RVM injection sites for animals that received muscimol and either DAMGO plus DPDPE (□) or QX-314 (■).

Figure 7A

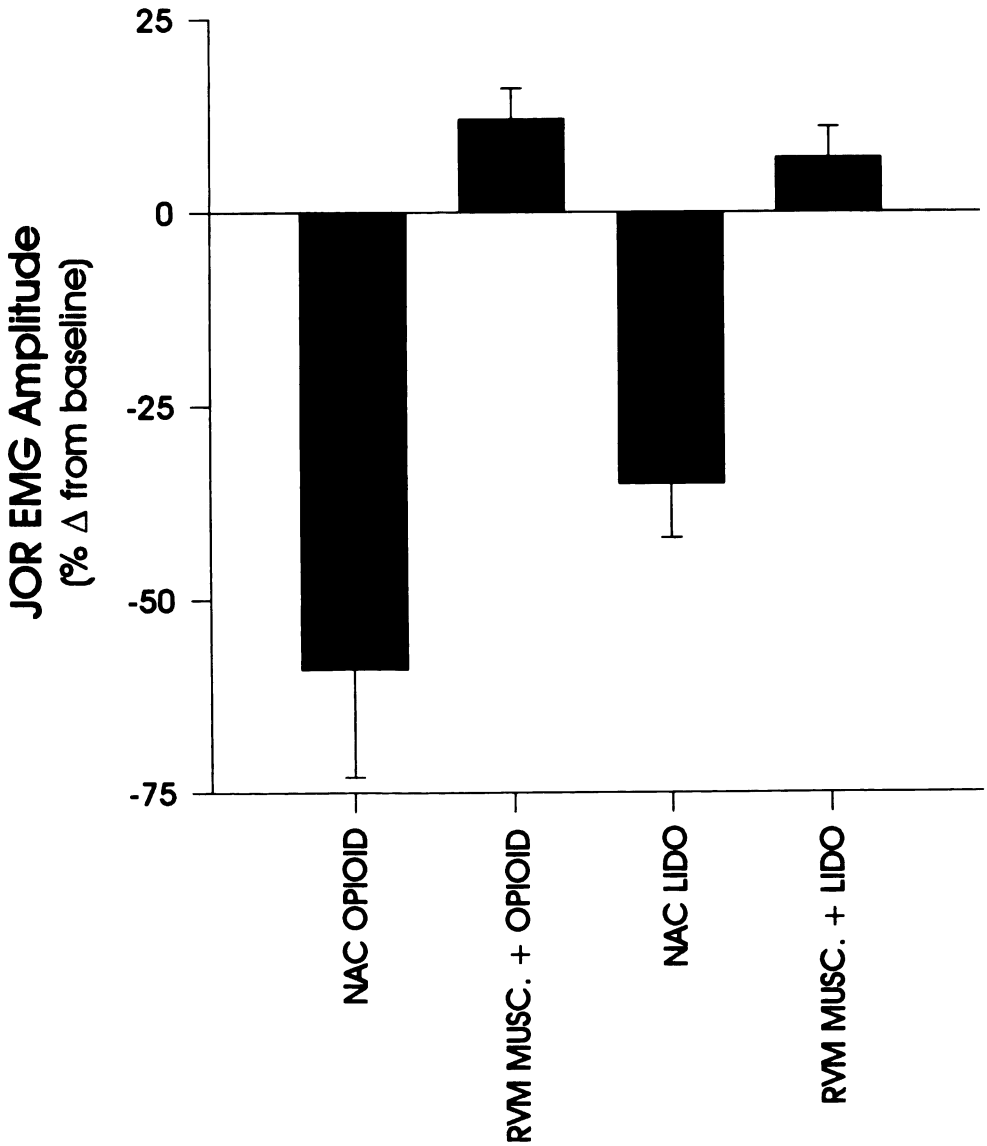

JOR EMG Amplitude
(% Δ from baseline)
25
0
-25
-50
-75
NAC OPIOID
RVM MUSC. + OPIOID
NAC LIDO
RVM MUSC. + LIDO

Figure 7B/C

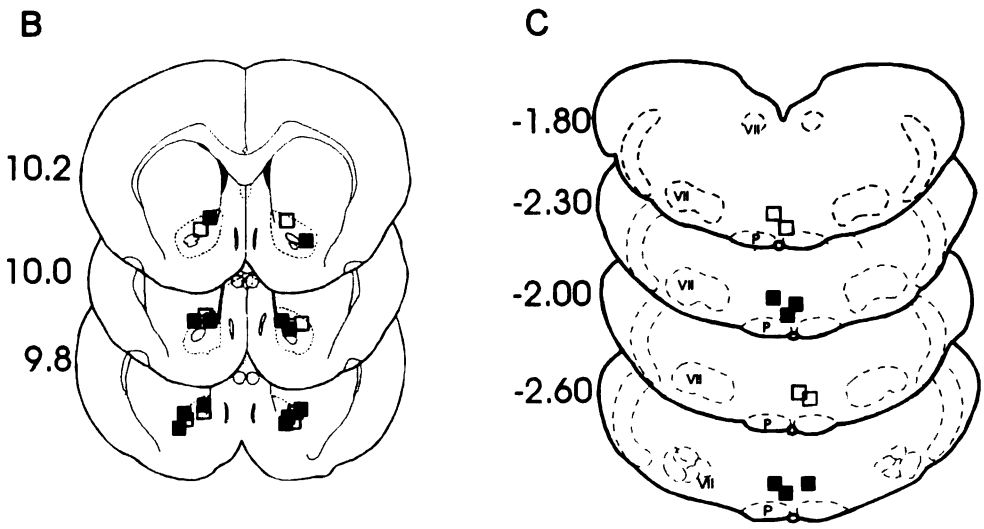


Figure 8.

A. The effect of naloxone injected into the RVM on attenuation of the JOR produced by either DAMGO plus DPDPE or lidocaine injected into the NAC. Either DAMGO plus DPDPE (○), 45 ng of each opioid in 0.3 μl CSF per side, n = 5, or lidocaine (●), QX-315, 333 ng in 0.3 μl H_2O per side, n = 4, was injected into the NAC 10 min after bilateral injection of naloxone methiodide, 1 μg in 0.5 μl 0.9% saline, into the RVM. Repeated measures ANOVA with one between subjects factor (treatment) and one within subjects factor (time) demonstrated no significant difference between these two groups and the two groups that received these same NAC treatments in the absence of RVM naloxone (see Fig. 3).

B. NAC injection sites for animals that received RVM naloxone methiodide and either DAMGO plus DPDPE (○) or lidocaine (●).

C. RVM injection sites for animals that received RVM naloxone methiodide and either DAMGO plus DPDPE (○) or lidocaine (●).

Figure 8A

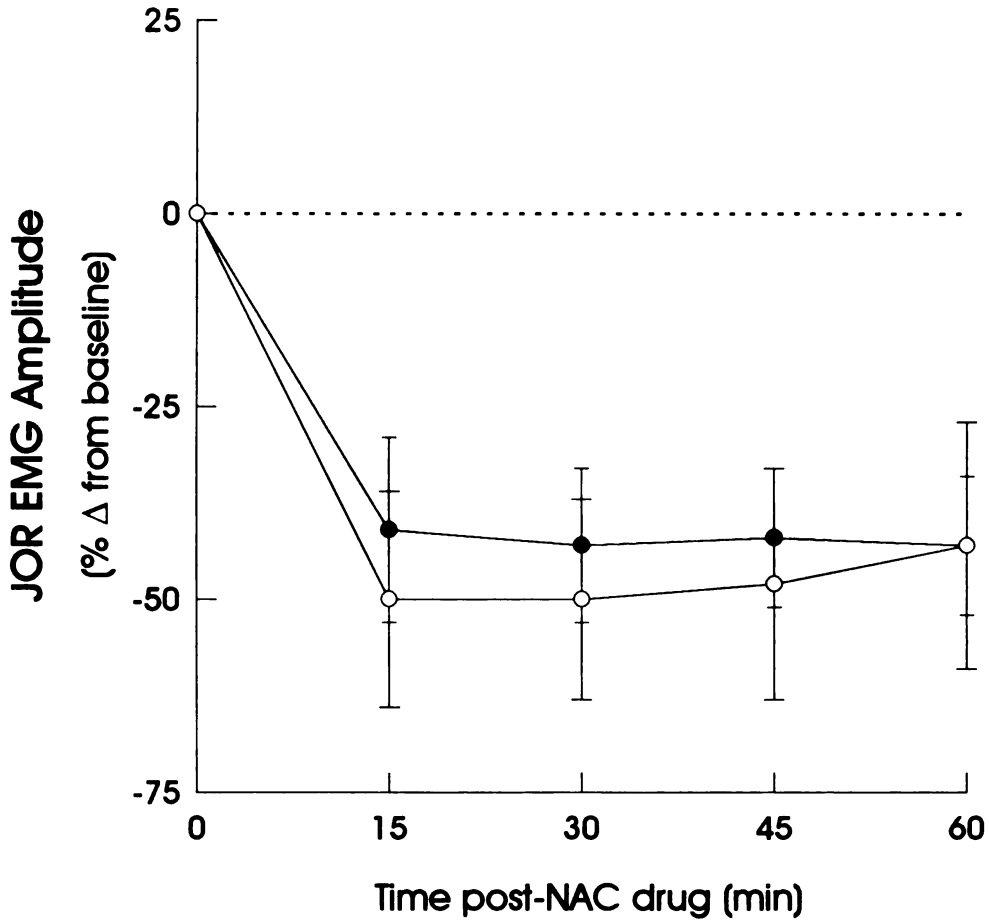

25
0
-25
-50
-75
JOR EMG Amplitude
(% Δ from baseline)
0
15
30
45
60
Time post-NAC drug (min)

Figure 8B/C

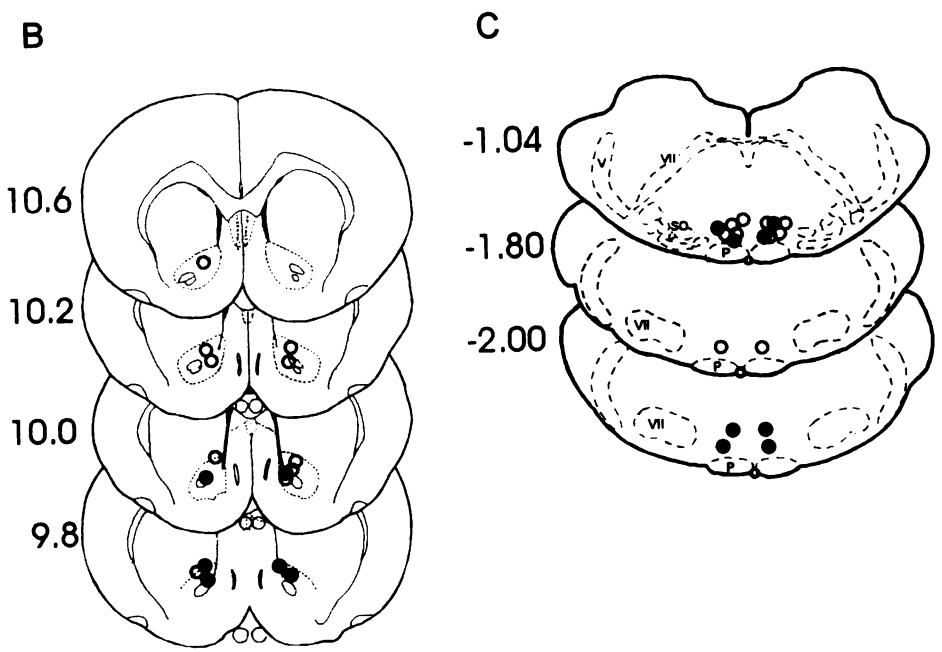


Figure 9.

A. The effect of injection of naloxone into the RVM on attenuation of the JOR by injection of DAMGO into the PAG. DAMGO, 30 ng in 0.3 μl CSF per side, was administered bilaterally to the ventrolateral PAG 10 minutes after bilateral onsite injection into the RVM of either vehicle (△), 0.5 μl 0.9% saline, n = 6, or naloxone methiodide (▲), 1 μg in 0.5 μl 0.9% saline per side, n = 6. DAMGO, 60 ng in 0.3 ml CSF, single injection, 12° angle, was administered to ventrolateral PAG 10 minutes after bilateral offsite injection into the RVM of naloxone methiodide (O), 1 μg in 0.5 μl 0.9% saline per side, n = 5. Repeated measures ANOVA with one between subjects factor (treatment) and one within subjects factor (time) demonstrated significant difference between the groups. Fisher's posthoc analysis showed the group that received onsite naloxone methiodide was significantly different from the groups that receive either offsite naloxone methiodide or vehicle injected into the RVM. These latter two groups were not significantly different from each other.

B. PAG injection sites for animals that received DAMGO injected into the PAG and either vehicle (△), onsite naloxone methiodide (▲), or offsite naloxone methiodide (O) injected into the RVM.

C. RVM injection sites for animals the received DAMGO injected into the PAG and either vehicle (△), onsite naloxone methiodide (▲), or offsite naloxone methiodide (O) injected into the RVM.

Figure 9A

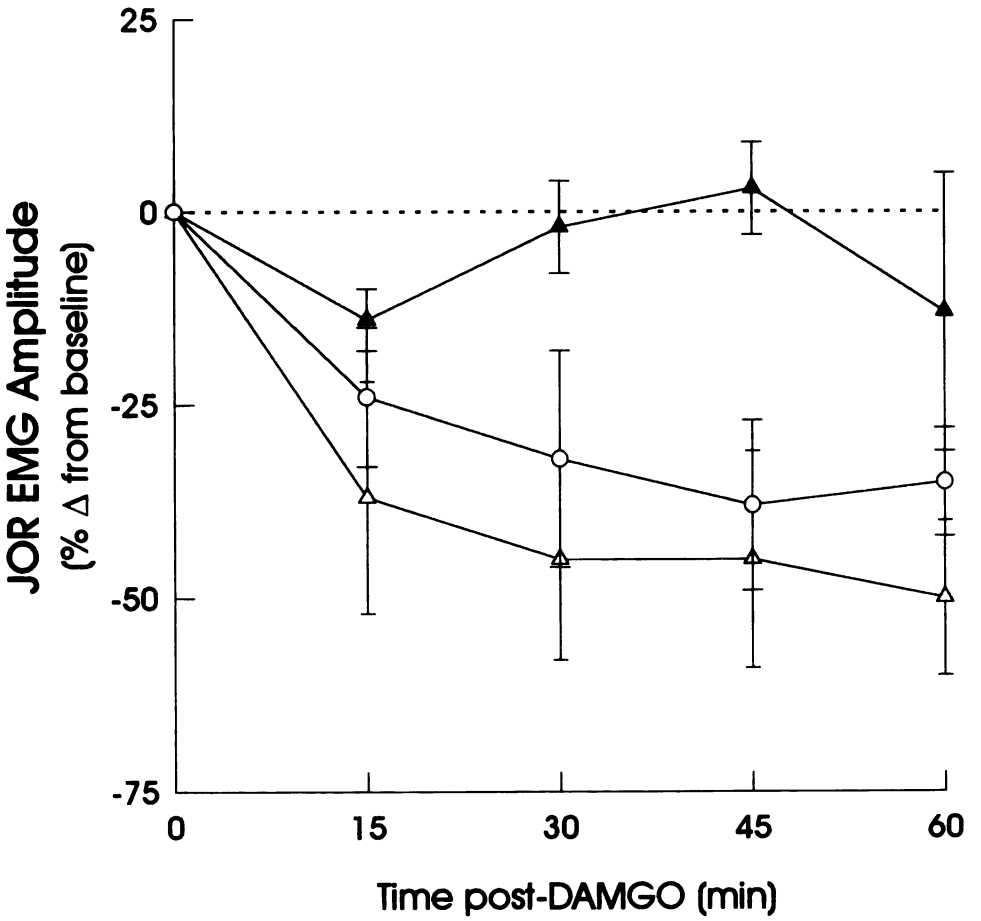
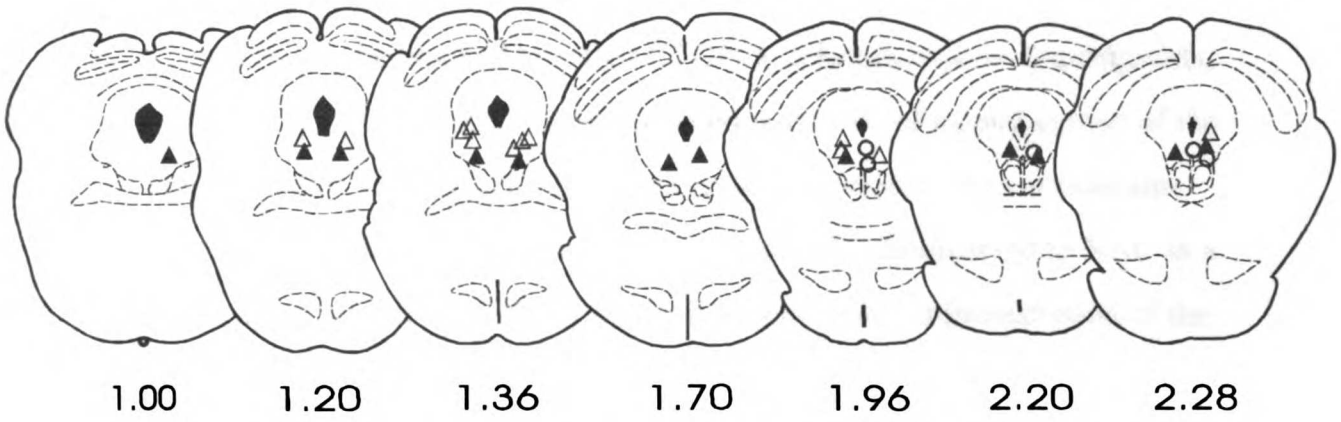

25
0
-25
-50
-75
JOR EMG Amplitude
(% Δ from baseline)
0
15
30
45
60
Time post-DAMGO (min)

Figure 9B/C

B



C

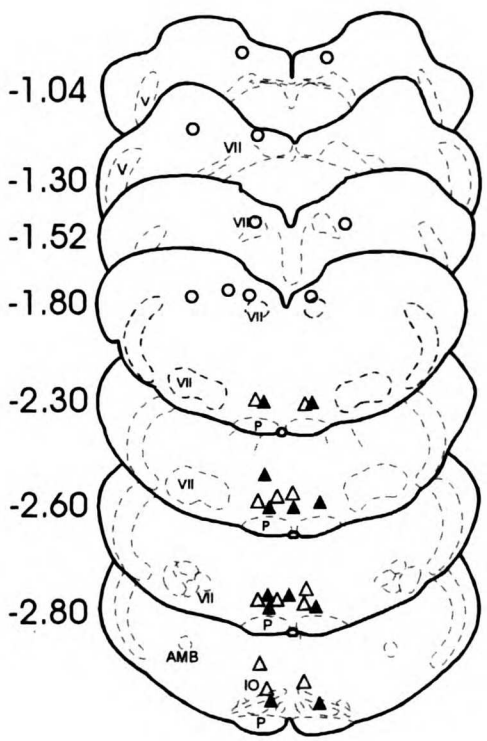
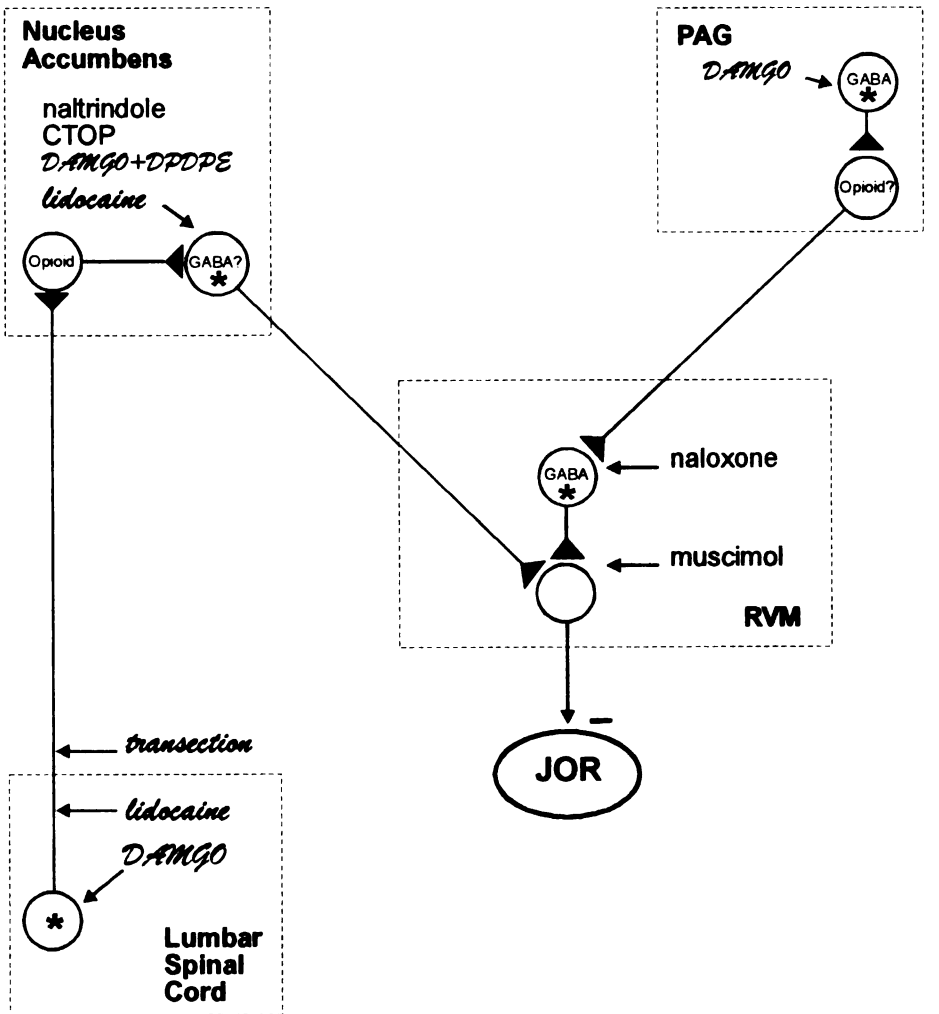


Figure 10.

Schematic diagram depicting the main findings of this research. *Lumbar spinal cord:* Either i.t. DAMGO, i.t. lidocaine or spinal cord transection at T_5T_6 attenuate the JOR. *Nucleus accumbens* Attenuation of the JOR by these spinal treatments can be blocked by either CTOP or naltrindole(μ- or δ-antagonists, respectively). Furthermore, administration into NAC of a combination of the opioids DAMGO plus DPDPE (μ- or δ-agonists, respectively) or lidocaine attenuates the JOR. Neither DAMGO nor DPDPE administered to NAC as a single agent affects the JOR. *Rostral ventral medulla:* administration of the $GABA_A$ receptor agonist muscimol blocks attenuation of the JOR by the spinal or NAC treatments shown as well as by DAMGO injected into the PAG. Naloxone, however, blocks only attenuation of the JOR by DAMGO administered into the PAG.

Figure 10



Legend

script: attenuates JOR
plain text: blocks attenuation of JOR

* = tonically active neuron
▲ = inhibitory synapse
– = antinociception (behavioral)

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