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Nucleus Accumbens Facilitates Nociception

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Abstract

We have previously demonstrated an opioid link in nucleus accumbens (NAc) that mediates antinociception produced by a novel ascending pain modulation pathway. For example, noxious stimulation induces heterosegmental antinociception that is mediated by both mu- and delta-opioid receptors in NAc. However, spinal intrathecal administration of the mu-receptor agonist [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO) also induces heterosegmental antinociception. The aim of the present study in the rat was to identify the intra-NAc opioid receptors that mediate the antinociceptive effects of spinally administered DAMGO and also to determine the effect of NAc efferent activity on nociception. Intra-NAc administration of either the mu-opioid receptor antagonist Cys², Tyr³, Orn⁵, Pen⁷amide (CTOP) or the delta-opioid receptor antagonist naltrindole blocked the antinociceptive effect of spinally administered DAMGO on the jaw-opening reflex (JOR). Injection of quaternary lidocaine (QX-314) attenuated the JOR, suggesting that the output of NAc is pronociceptive. In support of this, intra-NAc injection of the excitatory amino acid agonist kainate enhanced the JOR. Thus, it is possible to modulate activity in NAc to bidirectionally attenuate or enhance nociception, suggesting a potential role for NAc in setting nociceptive sensitivity.

Keywords

rat; pain; analgesia; opioids; mu; delta; lidocaine; glutamate; kainate; jaw opening reflex

Introduction

In a previous study we demonstrated that spinal intrathecal administration of the local anesthetic lidocaine or the μ -opioid receptor agonist [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO), or even spinal transection, all induce heterosegmental supraspinal antinociception in the trigeminal jaw-opening reflex (JOR) that can be blocked by intracerebroventricular administration of the non-selective opioid receptor antagonist naloxone (Gear and Levine, 1995). Based on this study we concluded that inhibition of

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neural activity in the spinal cord produces antinociception mediated by supraspinal endogenous opioids. Subsequently we demonstrated that noxious stimulation to the hind paw also inhibits neural activity in the spinal cord, inducing heterosegmental antinociception also mediated by endogenous opioids at a supraspinal site, specifically the nucleus accumbens (NAc) (Gear, et al., 1999, Tambeli, et al., 2002).

The aim of the present study was to further explore this ascending spinal-supraspinal analgesia circuit by determining the effect of its activation on efferent activity from NAc. If activation of intra-accumbens opioid receptors inhibits efferent activity from NAc, then NAc output would be pronociceptive. However, although opioids inhibit neural activity (Nicoll, et al., 1980), this inhibition has been shown in some cases to increase efferent activity from the site of opioid administration through a mechanism of circuit disinhibition (e.g., see review by (Fields and Basbaum, 1994). Therefore, to determine whether the opioid link in NAc increases or decreases efferent activity to produce analgesia, we compared the effect of exogenous opioids, QX 314 (a quaternary form of lidocaine) and the glutamate receptor agonist kainate on nociception, using the trigeminal jaw-opening reflex (JOR). We also identified the intra-NAc opioid receptors that mediate the antinociceptive effects of a spinally administered μ -opioid agonist.

Material and methods

Anesthesia

Experiments were performed in 250 – 450 g male Sprague-Dawley rats (n = 66, Bantin and Kingman, Fremont, CA) that were anesthetized with an intraperitoneal injection of 0.9 g/kg urethane and 45 mg/kg α -chloralose (both from Sigma-Aldrich, St. Louis, MO). This method provides a state of anesthesia with stable physiological parameters (Buelke-Sam, et al., 1978) and a stable JOR EMG signal (Gear and Levine, 1995) over the time period required to complete the experiments. All experimental protocols were approved by the University of California, San Francisco, Institutional Animal Care and Use Committee and conformed to National Institutes of Health *Guidelines for the Care and Use of Experimental Animals*. Effort was made to limit the number of animals used and their discomfort.

Electrode implantation

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

Nociceptive assay

Because the JOR is a nociceptive reflex only when the stimulus intensity is sufficient to activate nociceptors, the stimulation current was set at three times threshold to assure that this would be the case (Mason, et al., 1985). Each data point consisted of the average peak-to-peak amplitude of 12 consecutive jaw-opening reflex EMG signals evoked by stimulating the tooth pulp with 0.2 ms square wave pulses at a frequency of 0.33 Hz. Pre-intervention baseline amplitude was defined as the average of the last three data points, recorded at 5-minute intervals, before an experimental intervention. As is customary for JOR studies (Ahn, et al., 1998, Banks, et al., 1992, Belforte, et al., 2001, Chiang, et al., 1990, 1991, Gear, et al., 1999, Gear and Levine, 1995, Schmidt, et al., 2001, Takeda, et al., 1998, Zhang, et al.,

1998, 1999), data were normalized for differences in baseline by calculating the percentage change from baseline for each post-intervention data point. These values were used in the statistical analyses and were also plotted (mean $\pm$ s.e.m.) in the figures such that JOR attenuation is represented on the y-axis as positive numbers (i.e., antinociception); JOR enhancement (i.e., hyperalgesia) is represented as negative numbers. In all figures the x-axis represents the time in minutes following the first (or only) experimental intervention, which, in most cases, was an i.t. injection.

Drug administration

Intrathecal (i.t.) administration of drugs to the lumbar region of the spinal cord was through a polyethylene catheter 10 μ l 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 injection 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 μ l syringe (Hamilton, Reno, NV). Supraspinal injection volumes were 0.3 μ l and were carried out over a period of 90 seconds; injection cannulae were left in place an additional 30 seconds after injection. Injection sites were verified by histological examination (50 μ m sections stained with cresyl violet acetate) and were plotted on coronal sections adapted from the atlas of (Paxinos and Watson, 1986). Areas of on-site and off-site injections are shown in Figure 1.

Drugs

[D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO), kainate, Cys², Tyr³, Orn⁵, Pen⁷ Amide (CTOP), and D-Pen^{2,5}-enkephalin (DPDPE) were dissolved in phosphate buffered saline. Naltrindole and lidocaine N-ethyl bromide salt (QX-314) were dissolved in distilled water. All drugs were purchased from Sigma, St. Louis, MO. QX-314 is a quaternary derivative of lidocaine used to retard drug spread from the site of injection (Schroeder, et al., 1991). To retard rostral flow of i.t. administered drugs, 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 μ l followed by 10 μ l of vehicle (equal to the volume of the i.t. catheter).

Statistical Analysis

To determine if there were significant differences between experimental groups, we employed two-way repeated measures ANOVA with one within-subjects factor (time) and one between-subjects factor (group). If there was a significant group $\times$ time interaction, indicating that the groups in the analysis demonstrated significantly different time courses, multivariate analyses (i.e., one-way ANOVAs with Scheffé post hoc analyses) were performed for all time points in order to determine which points accounted for the interaction. In these cases, Bonferroni correction was applied in order to account for multiple comparisons. For within-subjects effects the Mauchly criterion was used to determine if the assumption of sphericity was met; if not, Greenhouse-Geiser *p*-values are presented. If the main effect of group was significant and there were more than two groups, Scheffé post hoc analyses were used to determine the basis of the difference.

Results

Effect of NAc efferent activity on nociception

Intra-NAc lidocaine—In separate groups of rats QX-314 (333 ng) or vehicle was administered into NAc (see Fig. 1 for location of injections) to investigate the effect of the local anesthetic lidocaine on nociception (Fig. 2). In a third group QX-314 was injected into sites adjacent to the NAc (off-site injections). The JOR was attenuated following the on-site injection of QX-314, but not following off-site or vehicle injections, suggesting that inhibition of efferent activity from NAc by a local anesthetic produces antinociception.

Two-way repeated measures ANOVA showed a significant main effect of group ($F_{2,18}=24.010$; $p<0.001$); Scheffé post hoc analysis showed that the on-site QX-314 group differed significantly from the other two groups ($p<0.001$ in both cases), which did not differ significantly from each other. There was also a significant time $\times$ group interaction ($F_{8,72}=10.185$; $p<0.001$). Multivariate analysis showed that the QX-314 group differed significantly from the other two groups at each time point ($p=0.024$ at 15 minutes; $p<0.001$ at each of the other three time points).

QX-314 occlusion—We have previously (Schmidt, et al., 2002) shown that intra-NAc injection of a combination of the μ - and δ -opioid receptor agonists DAMGO and DPDPE induces antinociception. To determine if the antinociception resulting from intra-NAc injection of QX-314 and that produced by DPDPE plus DAMGO is mediated by the same mechanism, an occlusion experiment was performed. Injection of DPDPE plus DAMGO (150 ng each per side) into NAc was followed, 40 min later, by injection of QX-314 (333 ng per side) into the same sites. The effect on the JOR was measured thirty min after each intervention. The change in the JOR after both of these treatments was calculated as the change from the baseline recorded prior to the injection of the opioid combination. QX-314 did not significantly affect the JOR following the attenuation produced by the opioid combination (Fig. 3). These results support the suggestion that prior administration of the opioid combination occluded the ability of QX-314 to attenuate the JOR, and are consistent with the suggestion that opioids produce antinociception by inhibition of NAc efferent activity.

Intra-NAc kainate—To determine if increasing NAc efferent activity could enhance nociceptive responses, the excitatory amino acid agonist kainate (63 ng) or vehicle was administered bilaterally into NAc in separate groups of rats. Kainate induced significant enhancement of the JOR supporting the suggestion that increasing activity in NAc was pronociceptive (Fig. 4). These results, taken together with the lidocaine (QX-314) data (Fig. 2), support the suggestion that NAc can produce bidirectional modulation (i.e., enhancement or attenuation) of nociceptive responses.

The two-way repeated measures ANOVA showed a significant time $\times$ group interaction ($F_{4,36}=3.182$; $p<0.048$). Multivariate analysis showed that the kainate group differed significantly from the vehicle group at 15 and 30 minutes ($p=0.047$ and $p=0.036$, respectively) but not at 45 and 60 minutes ($p=0.379$ and $p=0.650$, respectively). The main effect of group was not significant ($F_{1,9}=3.287$; $p=0.103$).

NAc μ - and δ -opioid receptors mediate i.t. DAMGO-induced antinociception

In an earlier study (Schmidt, et al., 2002) we demonstrated that both μ - and δ -opioid receptors in NAc mediate the antinociceptive effect of noxious stimulation (i.e., subdermal capsaicin administration). To determine if these same opioid receptors also mediate the antinociceptive effect of i.t. DAMGO (7.5 μ g), separate groups of rats received intra-NAc

administration of either the μ -opioid receptor antagonist CTOP (1 μ g) or the δ -opioid receptor antagonist naltrindole (1 μ g). Five minutes later DAMGO was administered i.t. to the lumbar region of the spinal cord (Fig. 5). Both CTOP and naltrindole, but not their vehicles, blocked the ability of spinal DAMGO to attenuate the JOR, supporting the suggestion that the same intra-NAc circuit mediates the antinociceptive effects of both i.t. DAMGO and subdermal capsaicin.

Two-way repeated measures ANOVA showed a significant main effect of group ($F_{4,24}=8.434$; $p<0.001$); Scheffé post hoc analysis showed that the i.t. DAMGO alone group differed significantly from DAMGO/CTOP ($p=0.013$) and the DAMGO/naltrindole ($p=0.008$) groups but not the DAMGO/vehicle groups ($p=1.000$ and $p=0.986$).

Discussion

In this study we have produced evidence that: 1) pain modulation results from increasing or decreasing tonically active efferent pronociceptive efferent signals from NAc, 2) these signals can be decreased (i.e., induce antinociception) by endogenous opioids within NAc, and 3) the heterosegmental antinociceptive action of spinal DAMGO is mediated by both μ - and δ -opioid receptor inhibition of the output from NAc. It is unlikely that these effects resulted from the anesthesia because we have observed heterosegmental antinociception that could be blocked by intra-accumbens naloxone in awake animals (Gear, et al., 1999).

Efferent activity from NAc is pronociceptive

In previous studies (Gear and Levine, 1995, Tambeli, et al., 2002) we showed that treatments that inhibit neural activity in the spinal cord induce heterosegmental antinociception (i.e., antinociception that can be observed remotely from the site of spinal administration), and that this antinociception can be blocked by intra-NAc administration of the non-selective opioid receptor antagonist naloxone. In the current study, we extend these findings by showing that both μ - and δ -opioid receptors play a role, similar to the role played by these receptors in noxious stimulus-induced antinociception (Schmidt, et al., 2002). However, the mechanism by which intra-NAc μ - and δ -receptors induce antinociception has not been studied.

Opioids are known to inhibit neural activity (Nicoll, et al., 1980), but this inhibition could act either directly on efferent projection neurons, thereby decreasing efferent activity, or on upstream neurons that tonically inhibit projection neurons, thereby disinhibiting (i.e., increasing) efferent activity. If inhibition of projection neurons is the mechanism, intra-NAc opioid injection would produce an observable effect only if the efferent neurons are tonically active. If this is the case, injection of a local anesthetic should mimic the effect of the opioid. On the other hand, if the action of the opioid is to disinhibit efferent activity, injection of local anesthetic would not be expected to mimic the effect of opioids; instead, the local anesthetic would be expected to block the effect of the opioid. To distinguish between these two mechanisms, QX-314 (a quaternary derivative of lidocaine) was administered into NAc. QX-314 attenuated the JOR suggesting that antinociception results from inhibition of tonic efferent activity from NAc. The observation that prior administration of the μ -/ δ -opioid combination occluded the ability of QX-314 to further attenuate suggests that activation of NAc opioid receptors or administration of a local anesthetic produces antinociception by a similar mechanism (i.e., reduction of NAc efferent activity).

Bidirectional modulation of nociception by NAc

If inhibition of efferent activity from NAc produces antinociception, then, logically, NAc efferent activity is pronociceptive (i.e., facilitates nociception). To determine if the basal

pronociceptive efferent signal is maximal, the excitatory amino acid receptor agonist kainate was injected into NAc. Kainate enhanced the JOR suggesting that NAc efferent activity is pronociceptive and further supporting the suggestion that ongoing NAc efferent activity maintains the animal in a nociceptive state that can be modulated in either direction. That is, events that reduce NAc efferent activity from its basal level produce behavioral antinociception, whereas events that increase NAc efferent activity above its basal level produce hyperalgesia.

Although in this study we have not addressed the downstream mechanism by which NAc efferent output modulates nociception, it is well known that the rostral ventral medulla (RVM) is a major neural substrate for the descending pain inhibitory system, and studies have increasingly implicated the RVM in nociceptive facilitation as well as inhibition (see (Porreca, et al., 2002, Vanegas and Schaible, 2004) for reviews). For example, neuropathy induced by spinal nerve ligation has been shown to produce descending nociceptive facilitation mediated in RVM (Kovelowski, et al., 2000); RVM was also shown to play a facilitatory role in the persistent pain associated with pancreatitis (Vera-Portocarrero, et al., 2006) as well as fentanyl hypersensitivity (Rivat, et al., 2009) and by tolerance resulting from prolonged exposure to drugs like morphine, thereby degrading analgesic efficacy (Vanderah, et al., 2001). However, it has been suggested that the facilitatory influence does not originate in RVM, but from a —forebrain loop (Porreca, et al., 2002), and we have produced indirect evidence for a NAc – RVM circuit (Gear and Levine, 2009). Taken together, these findings raise the possibility that NAc may play an important role in nociceptive facilitation as well as antinociception.

Role of the spinal cord in controlling the efferent output of nucleus accumbens

In this and earlier studies (Gear and Levine, 1995, Tambeli, et al., 2002) we have demonstrated that inhibition of neural activity at the level of the spinal cord induces heterosegmental antinociception mediated by endogenous opioids in NAc, indicating that the spinal cord exerts control of NAc-mediated antinociception. Whether the reverse is true—that enhancing the spinal signal to NAc induces a pronociceptive effect—remains to be investigated, but the current finding that intra-NAc kainate enhances nociception supports the suggestion that such a mechanism is possible. If such a mechanism is found to exist, it would suggest that nociceptive state is subject to influence by NAc and, in turn, by ascending influences from the spinal cord.

In summary, we provide evidence that activation of endogenous opioids in NAc produce antinociception by inhibiting efferent activity. Importantly, enhancing NAc efferent activity facilitates nociception, raising the possibility that such modulation of activity in NAc could contribute to acute or chronic pain syndromes. Taken together with our recent observation that stress can attenuate NAc-mediated analgesia (Ferrari, et al., 2010), we propose that NAc may play a critical role in diverse conditions that produce pain and analgesia.

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Abbreviations

ANOVA	analysis of variance
CTOP	Cys ² ,Tyr ³ ,Orn ⁵ ,Pen ⁷ amide

DAMGO	[D-Ala ² ,N-Me-Phe ⁴ ,Gly ⁵ -ol]-enkephalin
DPDPE	D-Pen ^{2,5} -enkephalin
EMG	electromyographic
GABA	gamma amino butyric acid
i.t	intrathecal
JOR	jaw opening reflex
NAc	nucleus accumbens
QX-314	lidocaine N-ethyl bromide salt
RVM	rostral ventral medulla

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Figure 1. NAc injection sites. Filled circles indicate —on-site injections; open circles indicate —off-site injections. All injections were bilateral; some injection sites overlap others. Numbers indicate distance rostral to bregma (mm). (Adapted from (Paxinos and Watson, 1986).

Figure 2.

The effect of intra-NAc lidocaine administration on the JOR. QX-314 (333 ng, 0.3 μ l per side), a quaternary analog of the local anesthetic lidocaine, or vehicle (same volume) was administered into NAc. QX-314 was also administered off-site (same dose and volume). On-site, but not off-site, QX-314 significantly attenuated the JOR at all post-administration time points, indicating that in otherwise untreated animals NAc produces a pronociceptive signal, and that suppression of this signal by lidocaine results in antinociception as revealed by attenuation of the JOR.

Figure 3. Effect of QX-314 on analgesia induced by intra-NAc administration of exogenous opioids. The μ -opioid receptor agonist DAMGO (—Dgo" 150 ng) and the δ -opioid receptor agonist DPDPE (—Dpdpe 150 ng) were combined and administered to NAc ($n = 5$). The JOR was recorded thirty minutes later and then QX-314 (333 ng) was administered into the same site. Although QX-314 alone can attenuate the JOR (Fig. 2), its effect was occluded when administered subsequently to the opioid combination, suggesting that these two treatments produce analgesia by the same NAc mechanism, that is, attenuation of efferent activity.

Figure 4.

The effect of intra-NAc kainate administration on the JOR. The excitatory amino acid agonist kainate (63 ng, 0.3 μ l per side) or vehicle (same volume) was administered into NAc. Kainate significantly enhanced the JOR (shown as negative numbers on the y-axis) for at least thirty minutes after administration (asterisks), supporting the suggestion that NAc signals can modulate nociception bidirectionally; that is, can produce enhancement as well as attenuation.

Figure 5.

The effect intra-NAc opioid antagonists on antinociception induced by i.t. DAMGO administration. I.t. DAMGO (7.5 μ g, 15 μ l), a selective μ -opioid receptor antagonist, alone attenuated the JOR, an effect that was blocked by prior administration of CTOP (1 μ g, 0.3 μ l per side), a selective μ -opioid receptor antagonist, or naltrindole (1 μ g, 0.3 μ l per side), a selective δ -opioid receptor antagonist, but not their vehicles (same volumes).