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THE ROLE OF SPINAL OREXIN-1 RECEPTORS IN POSTERIOR HYPOTHALAMIC MODULATION OF NEUROPATHIC PAIN

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Abstract

The posterior hypothalamus (PH) is known to reduce nociceptive pain, but the effect of PH stimulation on neuropathic pain is not known. Because neurons containing the neurotransmitter orexin-A are located in the PH in some strains of rat and intrathecal injection of orexin-A produces antinociception in a neuropathic pain model, we hypothesized that orexin-A from neurons in the PH modifies nociception in the spinal cord dorsal horn. To test this hypothesis, the cholinergic agonist carbachol or normal saline was microinjected into the PH of lightly anesthetized female Sprague-Dawley rats with chronic constriction injury (CCI) and foot withdrawal latencies (FWL) were measured. Carbachol-induced PH stimulation produced dose dependent antinociception as shown by significantly increased FWL compared to saline controls. To investigate the role of orexin-A in PH-induced antinociception, the orexin-1 receptor antagonist SB334867 or dimethyl sulfoxide (DMSO) for control, was given intrathecally following carbachol-induced PH stimulation. SB334867 decreased FWL compared to DMSO controls. These data are suggestive that stimulating the PH produces antinociception in a neuropathic pain model and that the antinociceptive effect is mediated in part by orexin-1 receptors in the spinal cord dorsal horn.

Keywords

Antinociception; neuropathic pain; orexin-A; orexin-1 receptor; posterior hypothalamus

INTRODUCTION

Stimulation of the hypothalamus using electrical current or excitatory drugs like the non-specific cholinergic muscarinic agonist carbamyl choline (carbachol) can elicit marked antinociception (Klamt and Prado, 1991, Franco and Prado, 1996, Holden et al., 1999, Holden and Naleway, 2001, Holden et al., 2002, Leone et al., 2004, Holden et al., 2005). Electrical stimulation of the posterior hypothalamus (PH) produces potent antinociception in acute pain (Rhodes and Liebeskind, 1978), while chemical stimulation produces

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antinociception on the foot withdrawal and tail flick tests (Jeong and Holden, 2005) and reduces facial pain in rats (Bartsch et al., 2004). In addition, destruction or deactivation of the PH increases pain responses (Millan et al., 1983, Manning and Franklin, 1998). Human studies demonstrate that electrical stimulation of the PH produces antinociception in patients with primary headaches (Franzini et al., 2003, Leone et al., 2005, Rasche et al., 2006). These findings are suggestive that the PH plays a role in antinociception in both animals and humans in acute pain.

The effect of PH stimulation on neuropathic pain, which alters the way nociception is processed, is not known. However, preliminary studies done in our lab showed that PH stimulation produced antinociception in the chronic constriction injury (CCI) model (Jeong and Holden, 2006). The CCI model induces a peripheral mononeuropathy through loose ligation of the common sciatic nerve and produces pain responses in rats that resemble clinical neuropathic pain, including hyperalgesia and allodynia (Bennett and Xie, 1988, Attal et al., 1990, Bennett, 1993, Baron et al., 1999). The neurotransmitters involved in this PH-induced antinociception have not been identified.

One such neurotransmitter may be the hypothalamic peptide orexin. Originally determined to be a regulator of feeding behaviors, two forms of orexin, orexin-A and orexin-B, have been identified (Sakurai et al., 1998). Although derived from the same precursor, prepro-orexin, they have different structures, and preferentially bind and activate the orexin-1 receptor (OX₁R) and the orexin-2 receptor (OX₂R), respectively. Axons containing orexin-A and orexin-B, and the receptors OX₁R and OX₂R, are densely located in the superficial lamina in the spinal cord, which is involved in nociception (Craig and Dostrovsky, 1999, Millan, 1999, van den Pol, 1999, Date et al., 2000, Bingham et al., 2001, Hervieu et al., 2001, Cluderay et al., 2002, Grudt et al., 2002, Guan et al., 2004). Spinally-projecting orexin-containing neurons have been observed in the hypothalamus (van den Pol, 1999), and cell bodies of orexin-A neurons are located in the PH of Sprague-Dawley rats (Sakurai et al., 1998, Chen et al., 1999, Cutler et al., 1999, Nambu et al., 1999, Briski and Sylvester, 2001, Baldo et al., 2003, Cheng et al., 2003b). Intrathecal administration of orexin-A profoundly attenuates the increased pain response following nerve injury (Yamamoto et al., 2003a, Suyama et al., 2004, Kajiyama et al., 2005). These findings are suggestive that orexin, and particularly orexin-A, may also play a role in nociceptive processing.

We propose that orexin-A from neurons in the PH modifies nociception in the spinal cord dorsal horn in the CCI model of neuropathic pain. To test this hypothesis, we microinjected carbachol into the PH of rats with CCI. One group of rats received pretreatment with atropine sulfate to determine specificity of action of the carbachol. In separate groups of rats we stimulated the PH and gave the specific orexin-A receptor antagonist, SB-334867 intrathecally. Nociceptive responses were measured using the foot withdrawal test, a valid and reliable test of nociception (Yeomans and Proudfit, 1994) that measures response times to a noxious thermal stimulus. These data have been published in part as an abstract (Jeong and Holden, 2006, 2007).

EXPERIMENTAL PROCEDURES

The Institutional Animal Care Committee at the University of Illinois at Chicago approved the experimental protocols used in this study. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 90-23). All efforts were made to minimize animal suffering, reduce the numbers of animals used, and use alternatives to *in vivo* experiments.

Design

The pre-posttest experimental design with control groups was used for a series of behavioral experiments to test the functional effects of orexin-A in the PH on neuropathic pain in the spinal cord dorsal horn. Rats were randomly assigned either to the treatment or control group. Nociceptive modulation was manipulated through intracranial and intrathecal injections and outcomes measured through nociceptive testing using the foot withdrawal test.

Sample

Eighty-nine adult female Sprague-Dawley rats (230–300 gm; Charles River, Portage, MI) were used for behavioral experiments. All rats were maintained on a 12-hour light-dark cycle with free access to food and water. Each rat was only used once. To reduce the possibility of estrous cycle influence, rats were randomly assigned to either the treatment or control group, and no two rats were taken from the same cage on the same day.

Procedures

Surgical procedure for CCI—All rats received either CCI or sham surgery as follows: following deep pentobarbital anesthesia (50 mg/kg; intraperitoneal injection), and using aseptic technique, the common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue and four ligatures (4.0 chromic gut), spaced 1 mm apart, were tied loosely around the nerve. The length of the nerve affected was 4 to 5 mm long. The ligatures were placed in a manner that lightly constricted the diameter of the nerves when viewed with 40X magnification, which retards but does not arrest circulation through the superficial epineural vasculature, and produces a small, brief twitch in the muscle surrounding the exposure. The incision was closed in layers. Rats were observed daily for signs of neuropathic pain, including limping and guarding of the affected limb, and for lack of grooming, social inactivity, and weight loss of greater than 20%. The procedure of sham surgery was identical except that ligation of sciatic nerve was not done.

Analgesiometric testing—Pain responses were measured by the foot withdrawal test. The hairy surface of the hind feet was blackened with India ink to facilitate more uniform heating of the skin surface and a focused beam of high intensity light was directed at the lateral aspect of the hind paws. The time interval between the onset of skin heating and withdrawal was measured electronically. The cutoff was 8 seconds to prevent burning of the skin. The low cutoff was 1 second. Foot withdrawal latencies were then measured at 5-minute intervals for 50 minutes after microinjection or until they return to baseline.

Experiment 1: Stimulation of the PH—Two weeks after neuropathic surgery when peak neuropathic responses were observed, rats were lightly anesthetized with intraperitoneal pentobarbital (35 mg/kg) and immobilized in a stereotaxic frame after shaving the scalp. The midline scalp was infused with bupivacaine (0.25%; 0.10 ml, which provides local anesthetic relief for approximately 24 hr; (James Artwhol, DVM, University of Illinois at Chicago Biological Resources Laboratory, personal communication). An incision was made along the midline of the scalp, and a burr hole was drilled into the skull to allow insertion of a microinjection guide cannula into the PH to a location defined by the following stereotaxic coordinates: –3.1 mm from bregma, lateral –0.5 mm, vertical +2.1 mm, incisor bar set at –2.5 mm. A 23-gauge stainless steel guide cannula was lowered into the region of the left PH through a burr hole to the predetermined position. A 32-gauge stainless steel microinjection cannula was connected to a 10 μ l syringe by a length of PE-10 polyethylene tubing. This tubing was filled with a solution of the non-selective cholinergic

agonist, carbamylcholine chloride (carbachol; Sigma, St. Louis, MO, USA) which is also known to depolarize orexin neurons (Yamanaka et al., 2003, Bayer et al., 2005). Carbachol was dissolved in physiological saline and filtered through a 0.2 μ l syringe filter prior to use. The injection cannula was then inserted into the guide cannula, and extended 1 mm beyond the cannula edge. After a baseline the foot withdrawal latency was taken and recorded at 1 minute prior to microinjection, the carbachol was microinjected in a volume of 0.5 μ l over a 1-minute period using an electronic syringe pump. The microinjector was left in place for an additional 60 seconds prior to removal to reduce the flow of drug up the guide cannula. For control, 0.5 μ l of normal saline was injected to the PH in place of carbachol.

To determine the optimal effect of carbachol on the PH, a dose response study was done in which carbachol was microinjected in a dose of either 62.5, 125, or 250 nmol in a volume of 0.5 μ l normal saline and foot withdrawal latencies measured. The 125 nmol dose was determined to provide optimum antinociception and was used in all other experiments. To determine receptor specificity for carbachol microinjected in the PH, a separate group of rats was pretreated in the PH with the muscarinic antagonist, atropine sulfate (14 nmol/0.5 μ l normal saline; Sigma, St. Louis, MO, USA) to block the cholinergic effect. One minute later, the optimal dose of carbachol was microinjected into the PH. Foot withdrawal latencies were measured as described previously.

Experiment 2: The role of orexin in PH-induced antinociception—To determine whether the antinociceptive effect of PH stimulation was mediated by spinally projecting orexin-A neurons, an intrathecal catheter was inserted through an incision in the cisterna magna of rats and the tip position over the lumbar enlargement of the dorsal spinal cord (Yaksh and Rudy, 1976), as follows: rats were anesthetized with pentobarbital (35 mg/Kg) and mounted in a stereotaxic instrument. A midline incision was made over the occiput of the skull, extending from a line between the ears to a point approximately 2 cm caudal and a small incision was then made in the atlanto-occipital membrane. Keeping the angle of the catheter parallel with the dorsal surface of the brainstem, a 32-gauge intrathecal catheter was carefully advanced to a position 8 cm caudal of the cisterna at the level of lumbar enlargement (Yaksh and Rudy, 1976). The cannula was connected by PE-50 tubing to a 100 μ l syringe in an electronic syringe pump. The rats were then prepared for intracerebral injection as described above. A baseline foot withdrawal latency was taken, and 1 minute later the optimal dose of carbachol or normal saline was microinjected into the PH. Three foot withdrawal measurements were taken at 5-minute intervals. One of three doses of the orexin-1 receptor antagonist, SB-334867, 15, 30, or 60 nmol in a total volume of 15 μ l of dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) was then administered intrathecally over 1 minute and withdrawal latencies were measured 1 minute postinjection, and then every 5 minutes until the latencies returned to baseline (Yamamoto et al., 2002, Cheng et al., 2003a, Yamamoto et al., 2003b)

Additional groups of neuropathic rats were prepared only for intrathecal cannulation as described above. A baseline measurement was taken. One minute later, either 15, 30, or 60 nmol of SB-334867 in 15 μ l of DMSO was injected intrathecally similar to experiment 2, but without PH activation. Fifteen μ l of DMSO was used as control. Foot withdrawal latencies were then measured at 5-minute intervals for 50 minutes.

Histology—At the end of each experiment, the animals were deeply anesthetized with sodium pentobarbital. The brains were removed and placed in a formalin fixative overnight, followed by 20% sucrose solution. Microinjection sites relative to the PH were verified by cutting 40- μ m transverse brain sections, rinsed three times in cold phosphate buffered saline (PBS; 10 mM), mounted on gelatin-coated slides, stained with 0.05% neutral red, and coverslipped. Placement of the microinjection cannula was determined by plotting the most

ventral portion of the cannula tip in serial sections by brightfield microscopy. Tracings of the appropriate sections were then made using the NeuroLucida Imaging System (MicroBrightfield, Colchester, VT, USA) and compared to the coordinates described by Paxinos and Watson (1998). The data were excluded when the cannula was not in the PH. For nerve histology, the sciatic nerve was exposed after completing experimental procedures and observed for two constrictions or a region of nearly uniform thinning, a pronounced swelling just proximal to the constricted region, and a distinct, but smaller, swelling distally.

Statistical analysis—Data were analyzed using the computer program SPSS/PC, version 15.0 for Windows. To determine whether the neuropathic paw manifested thermal hyperalgesia, the foot withdrawal latencies difference between the neuropathic and the sham paws was tested two-way repeated measure analysis of variance (ANOVA). The level for statistical significance was set at $\alpha = 0.05$. Statistical comparisons of the left foot withdrawal latencies among treatment and control groups across time points were made using means and standard errors of the means, and two-way repeated measure ANOVA. Dunnett's test was used for post hoc comparisons.

RESULTS

Of the 89 rats used, 19 were excluded. Three rats showed self-mutilation; five rats did not exhibit neuropathic behaviors on the left affected foot; and 11 rats showed misplacement of either intracranial, intrathecal, or both cannulae.

Neuropathic pain behaviors

Rats that received neuropathic surgery on the left hind paw showed limping and a mild degree of foot-drop on the affected paw. Rats also raised the affected hind paw from the floor and held it in a protected position, similar to that observed by Bennett and Xie (1988). There was neither weight loss nor general weakness. To confirm a decrease in foot withdrawal latency on the left neuropathic paw as an indication of hyperalgesia, we compared the baseline latencies between the neuropathic and sham paws. As shown in Figure 1, the left neuropathic paw showed significantly shorter latencies compared to the sham paw ($p < 0.05$; 2.06 ± 0.07 and 3.46 ± 0.09 , respectively), indicating the presence of hyperalgesia in the affected paw. We also observed significantly decreased response latencies on the right, unligated paw for CCI rats compared to the sham right paw (2.60 ± 0.16 and 3.48 ± 0.12 sec, respectively). Therefore, we used separate groups of rats as sham surgery controls rather than using the unoperated right paw of CCI rats as control.

The effect of PH stimulation on neuropathic pain

To determine whether carbachol microinjection into the PH produces antinociception in neuropathic pain, carbachol or saline was microinjected into the PH at the locations shown in Figure 2 and the foot withdrawal latencies were measured. Because other areas in the brain are known to produce antinociception, we excluded data from those rats with microinjections outside the PH.

Figure 3 shows the dose response for three carbachol doses, taken at 10 minutes post injection, the optimal time of effect. Both the 125 and 250 nmol doses of carbachol significantly increased the foot withdrawal latencies as compared to saline controls ($p < 0.05$) while 62.5 nmol of carbachol did not significantly increase latencies ($p > 0.05$). We chose the 125 nmol dose for use in further experiments because it was the smallest dose that produced the greatest effect.

In rats with CCI ligation, microinjection of 125 nmol carbachol in the PH produced a significant increase in nociceptive responses compared to rats receiving saline in the PH (Figure 4; 7.9 ± 0.1 and 2.5 ± 0.2 sec, respectively, $p < 0.05$). This carbachol-induced antinociceptive effect occurred within 5 min post injection, went almost to cut off, and was maintained for over 50 min. This antinociceptive effect was blocked by pretreatment of the PH with atropine sulfate. As seen in Figure 4, foot withdrawal latencies in those rats receiving atropine were not different from rats in the saline control group ($p > 0.5$), indicating that carbachol acts at muscarinic receptors to produce PH-induced antinociception.

The effect of intrathecal administration of the OX₁R antagonist, SB-334867, on PH-induced antinociception in neuropathic pain

To determine whether PH-induced antinociception was mediated by orexin-A acting on OX₁R in the dorsal horn, carbachol (125 nmol/0.5 μ l of normal saline) was microinjected into the PH following a baseline measurement at -15 minutes and antinociception was obtained. The OX₁R antagonist, SB-334867, was then injected intrathecally in doses of 15, 30, or 60 nmol in 15 μ l of DMSO. DMSO was used as control. All doses of SB-334867 significantly decreased the foot withdrawal latencies as compared with DMSO controls ($p < 0.05$; Figure 5). Both the 30 and 60 nmol of SB-334867 decreased foot withdrawal latencies to the baseline level, suggesting that these doses both completely blocked antinociception induced by PH stimulation with carbachol. The 15 nmol dose also significantly decreased withdrawal latencies, but not to the level of controls, suggesting that only a partial blockade of PH-induced antinociception occurred at this lowest dose.

To verify that blockade of carbachol-induced antinociception by SB-334867 was not due to a volume injected in the intrathecal space, and to determine whether the orexin-A pathway is tonically active in the CCI model of neuropathic pain, SB-334867 was injected intrathecally in CCI rats without prior PH stimulation. None of the three doses of SB-334867 produced a significant difference in left foot withdrawal latencies compared to controls ($p > 0.05$).

DISCUSSION

In the present study, we demonstrated that carbachol-induced PH stimulation produced a robust antinociceptive response in female rats with CCI compared to rats given saline in the PH. This antinociceptive response was mediated by cholinergic receptors in the PH, and blocked by intrathecal administration of the OX₁R antagonist, SB-334867. While these findings are novel and are suggestive of a direct projection from orexin-containing neurons in the PH to the dorsal horn, we cannot say with certainty that such a connection exists in female Sprague Dawley rats. A number of studies identify orexin-immunoreactive neurons in the PH (Sakurai et al., 1998, Chen et al., 1999, Cutler et al., 1999, Nambu et al., 1999, Briski and Sylvester, 2001, Baldo et al., 2003, Cheng et al., 2003b), but while spinally projecting orexin-immunoreactive neurons have been identified in the LH (van den Pol, 1999), there is no anatomical evidence to date that shows definitive projection of PH orexin-containing neurons to the dorsal horn. It is also possible that carbachol in the PH stimulated non-orexin neurons that project to orexin-containing neurons elsewhere, such as the lateral hypothalamus (Baldo et al, 2003; Briski and Sylvester, 2001, vanden Pol, 1999), which could then project to the dorsal horn. Further anatomical studies are needed to determine the mechanisms involved in PH-induced antinociception.

Our use of female Sprague-Dawley rats in this study is meaningful because no reports that examine the role of orexin in female neuropathic pain exist in the literature. Two studies used male rats (Yamamoto et al., 2003a, Kajiyama et al., 2005) while one study did not state the sex of the rats that they used (Suyama et al., 2004). Researchers have a tendency to

avoid using females because of a possible effect of estrous cycle on variability (Mogil and Chanda, 2005), although variance between subjects in male rodents is also possible. We attempted to control for between group variability induced by estrous cycle in this study by random assignment to either treatment or control group and random testing through the rat estrous cycle. However, we did not determine estrous cycle and cannot completely rule out the effect of estrus on our findings. Further work to determine this possibility, as well as male/female differences, needs to be done.

We observed thermal hyperalgesia on the left CCI paw of lightly anesthetized female Sprague-Dawley rats compared to the left sham paw, a finding consistent with those of others who have used female (Jevtovic-Todorovic et al., 1998, Wagner et al., 1998) and male Sprague Dawley rats with the CCI model (Bennett and Xie, 1988, Mao et al., 1995, Ren et al., 1995, Yamazaki et al., 2001, Hama, 2002, Ro et al., 2004). Our baseline measurement for the present study was several seconds lower than that of other studies. We used a lower baseline in order to make comparisons with our acute studies that use similar baseline latencies. While it is possible that this lower baseline produced artificially flattened response latencies in rats with CCI, we do not think that is the case. Our cutoff for the low measurement was 1 sec. The mean foot withdrawal measurement for left ligated rats was 2.13 with a small standard error. This finding does not suggest a flattened baseline reading. Furthermore, while we showed a significant decrease in withdrawal latencies for left ligated rats as compared to controls, our purpose was not to measure the hyperalgesic effect of sciatic nerve ligation per se, but to show antinociception in rats with sciatic nerve ligation. While our baselines may not be comparable to those of other investigators, they show a hyperalgesic response in CCI rats, and are appropriate for the present study.

Bennett and Xie (1988) indicated that the CCI model does not affect the contralateral paw in male Sprague-Dawley rats and that each animal can serve as its own control. In our experiments, the baseline foot withdrawal latency of the contralateral paw was longer than that of the ipsilateral paw, but shorter than the sham group (Figure 1). The difference between the left and right CCI baselines is only about 0.5 second, and while statistically significant, it is unclear whether this finding has behavioral significance. Immunocytochemical changes have been observed in the dorsal horn cord contralateral to the side of CCI ligation, including the neuronal activation marker Fos, as well as the transcription factor cyclic adenosine monophosphate response element binding protein, which is thought to be involved in central sensitization. These markers are significantly increased in the contralateral spinal cord dorsal horn, although significantly less so as compared to the ipsilateral side (Kajander et al., 1996, Yamazaki et al., 2001, Song et al., 2005). Other studies indicate that the contralateral paw in the CCI model shows no increased response to a pain stimulus, even in the presence of immunocytochemical changes in the contralateral dorsal horn (Mao et al., 1995, Whiteside and Munglani, 2001, Leiphart et al., 2003, Ro et al., 2004). Because it is not clear whether ligating the left sciatic nerve in the CCI model actually affects the contralateral paw, we chose to use a separate group of control rats rather than using each rat as its own control.

To identify the role of dorsal horn OX₁R in antinociception produced by PH stimulation in neuropathic pain, we used a selective OX₁R antagonist, SB-334867. Our findings agree with Kajiyama and colleagues (2005), who showed that 50 µg of SB-334867 given intrathecally block antinociception in diabetic neuropathic pain, and others who blocked orexin-A induced antinociception in incision and inflammatory pain with 30 nmol of SB-334867 (Yamamoto et al., 2002, Cheng et al., 2003a, Yamamoto et al., 2003b).

The exact mechanisms of orexin-A and OX₁R in the spinal cord in pain modulation are not yet clear. Orexin-A is Gq protein coupled, increases synaptic activity and depolarizes

postsynaptic neurons (Sutcliffe and de Lecea, 2000, Eriksson et al., 2001, Kukkonen et al., 2002, Liu et al., 2002, Hoang et al., 2003, Dong et al., 2006) and therefore is likely to activate neurons. OX₁R are probably not on primary afferent terminals, excitatory interneuron, or projection neurons, which would increase nociceptive transmission when excited, a finding not in line with our observations (Figure 4). Based on our findings, OX₁R are more likely to activate GABA, glycine or enkephalin containing neurons in the dorsal horn which are inhibitory in nature (Tsuruhara and Takahashi, 1987, Atsumi et al., 1993, Fleming and Todd, 1994, Jonas et al., 1998, Kerchner et al., 2001, Kodama and Kimura, 2002, Liu et al., 2002, Moore et al., 2002, Baba et al., 2003, Peever et al., 2003, Dergacheva et al., 2005, Ataka and Gu, 2006). Although there is no evidence for colocalization of OX₁R on GABA, glycine, or enkephalin neurons to date, based on the antinociceptive role demonstrated in the present study, and the excitatory nature of orexin-A, it is likely that orexin-A neurons in the PH have an indirect inhibitory role on nociception in the dorsal horn by activating inhibitory interneurons, which then inhibit primary afferent terminals or projection neurons.

Intrathecal administration of the OX₁R antagonist SB-334867 given in the absence of PH stimulation produced no effect in our study. This finding is consistent with the work of others that show that the orexin-A pathway is not tonically active in neuropathic pain (Kajiyama et al., 2005), acute nociceptive pain (Bingham et al., 2001, Yamamoto et al., 2002), or inflammatory pain using carrageenan or formalin injection (Yamamoto et al., 2002, Yamamoto et al., 2003b). While intraperitoneal administration of SB-334867 alone increases hyperalgesic and allodynic effects in the mouse carrageenan model (Bingham et al., 2001), intraperitoneal injection introduces the possibility that peripheral OX₁R are involved and they may produce a different response than central receptors.

Implications for Clinical Practice

The findings of this study help to understand how the body produces endogenous analgesia. Ultimately, the results of this study could lead to the development of better pharmaceutical agents that make use of the endogenous systems and the development of non-pharmaceutical interventions that recruit the hypothalamus to activate the PH-orexin-A pathway.

CONCLUSION

In summary, PH stimulation with carbachol decreased CCI neuropathic pain as demonstrated by increased foot withdrawal latencies. Pretreatment with atropine sulfate blocked PH-induced antinociception. Intrathecal application of an OX₁R antagonist significantly decreased foot withdrawal latencies compared to DMSO controls, while the OX₁R antagonist given alone had no effect in neuropathic pain. These findings are suggestive that cholinergic muscarinic receptors mediate activation of PH neurons and activated PH neurons produced antinociception in part via a descending orexin-A pathway that may be direct or indirect. Following PH stimulation, orexin-A is released in the spinal cord dorsal horn and binds to spinal OX₁R to produce antinociception, probably through actions on inhibitory interneurons.

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List of Abbreviations

ANOVA	Analysis of Variance
CCI	Chronic Constriction Injury
DMSO	Dimethyl Sulfoxide
GABA	Gamma-Aminobutyric Acid
LH	Lateral Hypothalamus
OX₁R	Orexin-1 Receptor
OX₂R	Orexin-2 Receptor
PBS	Phosphate Buffered Saline
PH	Posterior Hypothalamus

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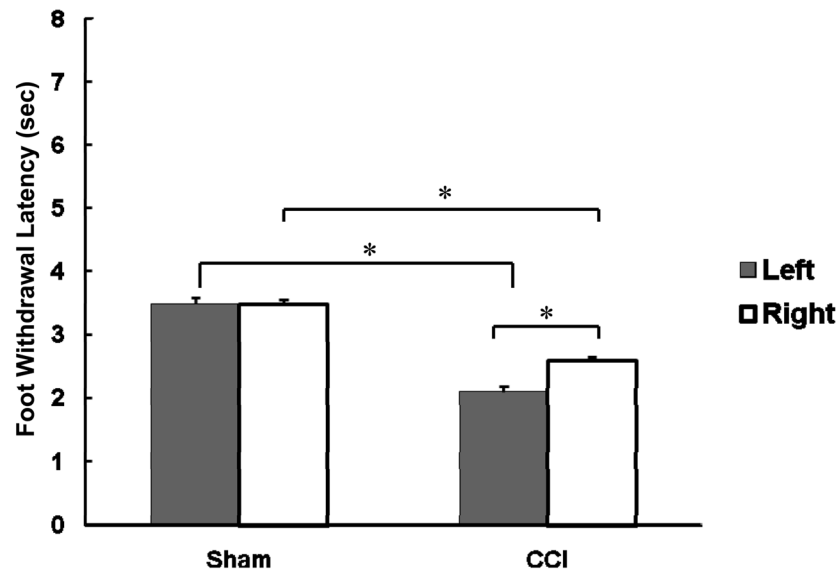


Figure 1. Comparisons of baseline foot withdrawal latencies between sham surgery and CCI groups for the left and right paws. The asterisk (*) shows statistically significant difference between groups ($p < 0.05$).

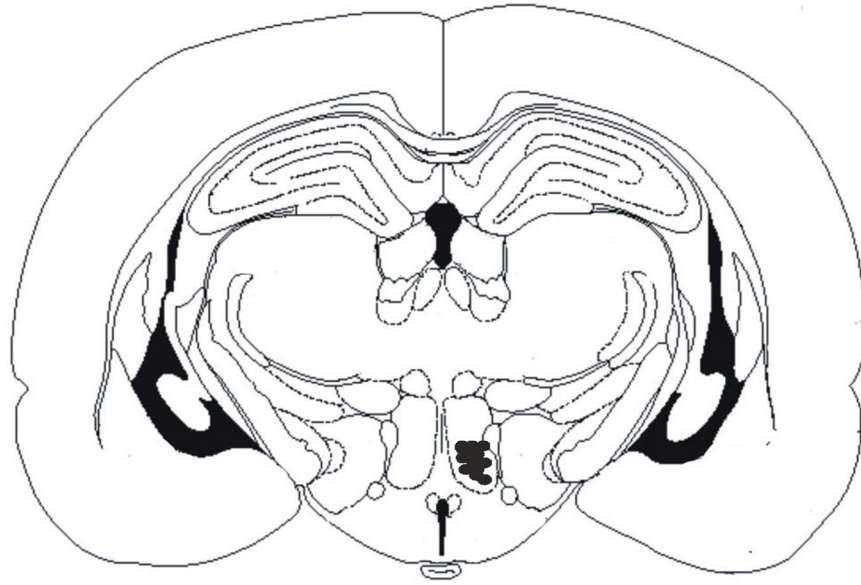


Figure 2.

Representative sample of microinjection sites in CCI rats receiving 125 nmol of carbachol. Mean response latencies were taken 10 minutes post injection and are represented as follows: filled circle, 6.1–8.0 seconds; open square, 4.1–6.0 seconds; open reverse triangle, 2.1–4.0 seconds. Abbreviations: 3V, 3rd ventricle; CA1, field CA1 of hippocampus; CA2, field CA2 of hippocampus; CA3, field CA3 of hippocampus; D3V, dorsal 3rd ventricle; f, fornix; LH, lateral hypothalamic area; LV, lateral ventricle; PH, posterior hypothalamic area. Stereotaxic coordinates are -3.1 mm from bregma, lateral -0.5 mm, vertical $+2.1$ mm, incisor bar set at -2.5 mm.

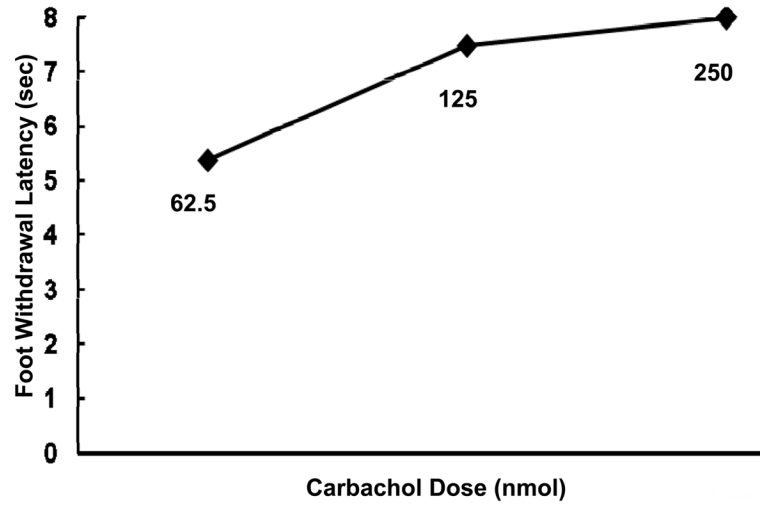


Figure 3. Foot withdrawal latencies of three different doses of carbachol on PH stimulation for the left CCI foot. Data indicate mean foot withdrawal latency of each dose at ten minutes post-injection.

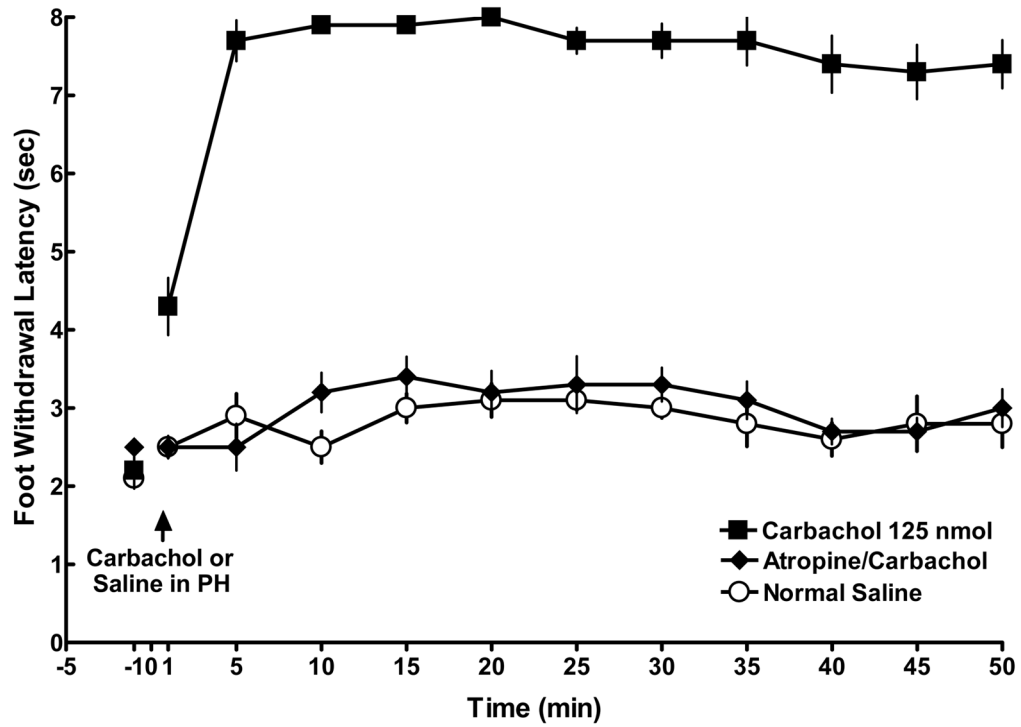


Figure 4.

The effect of carbachol-induced PH stimulation on the left CCI foot. Following a baseline response latency measurement at -1 minute, 125 nmol of carbachol (closed squares; $n = 5$ / group) was microinjected into the PH and produced robust antinociception compared to rats given normal saline (open circles; $n = 5$ / group) in the PH. Pretreatment with atropine sulfate at time -1 minute (closed diamonds; $n = 5$ / group) blocked carbachol-induced antinociception. Symbols equal the mean \pm SEM. Standard error bars may be obscured by the symbols.

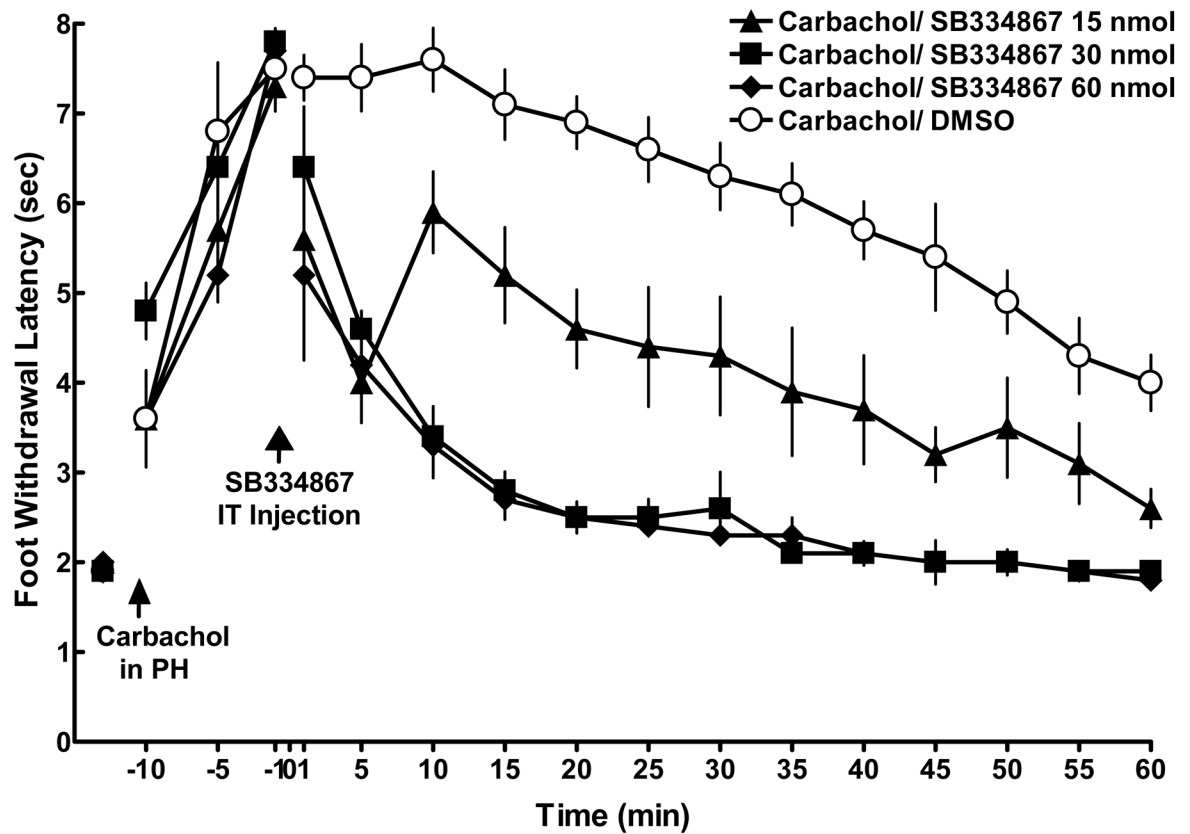


Figure 5. Intrathecal injection of OX_1R antagonist SB-334867 blocked PH-induced antinociception in CCI rats. Following a baseline measurement at -15 min, carbachol (125 nmol) was injected into the PH and three foot withdrawal latencies were taken at -10 , -5 and -1 minute. At time 0, the OX_1R antagonist, SB-334867 (15 , 30 , or 60 nmol; closed triangles, squares, and diamonds, respectively; $n = 5$ /group), was injected intrathecally. All three dose of SB-334867 produced a significant decrease in foot withdrawal latencies as compared to DMSO controls (open circles). Symbols equal the mean \pm SEM. Standard error bars may be obscured by the symbol.