Spinal Nociceptin Mediates Electroacupuncture-Related Modulation of Visceral Sympathoexcitatory Reflex Responses in Rats

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ABSTRACT

The role of nociceptin and its spinal cord neural pathways in electroacupuncture (EA)-related inhibition of visceral excitatory reflexes is not clear. Nociceptin/orphanin FQ (N/OFQ) is an endogenous ligand for a G-protein coupled receptor, called the N/OFQ peptide (NOP) receptor, which has been found to be distributed in the spinal cord. The present study investigated the importance of this system in visceral-cardiovascular reflex modulation during EA. Cardiovascular pressor reflex responses were induced by gastric distension in Sprague-Dawley rats anesthetized by ketamine and xylazine. Intrathecal injection of nociceptin (10 nM) at T1-2 attenuated the pressor responses by 35%, similar to the influence of EA at P 5-6 (42% decrease). Intrathecal injection of the NOP antagonist, [N-Phe\\(^1\)]-nociceptin (1-13) NH\(_2\) partially reversed the EA response. Pretreatment with the opioid receptor antagonist naloxone did not alter the EA-like inhibitory effect of nociceptin on the pressor reflex, while a combination of nociceptin receptor antagonist with naloxone completely abolished the EA response. Intrathecal injection of nociceptin attenuated the pressor responses to electrical stimulation of the rostral ventrolateral medulla by 46% suggesting that nociceptin can regulate sympathetic outflow. Furthermore, bilateral microinjection of NOP antagonist into either the dorsal horn or the intermediolateral column at T1 partially reversed the EA inhibitory effect. These results suggest that nociceptin in the spinal cord mediates part of the EA-related modulation of visceral reflex responses.

Keywords: NOP receptors, pressor response, dorsal horn, intermediolateral column, acupuncture
INTRODUCTION

Activation of sympathetic afferents during myocardial ischemia evokes excitatory cardiovascular reflexes including hypertensive responses and tachyarrhythmias, which can result in significant mortality (29, 32). These cardiovascular conditions potentially can be improved by acupuncture (5, 22, 40). Our previous studies have demonstrated that electroacupuncture (EA) at P 5-6 (Jianshi-Neiguan) acupoints, which refer to the pericardial meridian, overlying the median nerve in cats reduces the extent of myocardial ischemia during reflex increases in arterial blood pressure (BP) caused by stimulation of chemosensitive sensory nerve endings in the gallbladder (5, 22) and attenuates the reflex pressor responses to gastric distension in rats (9, 23, 41, 42). EA, through virtually identical pathways and neurotransmitter systems in the CNS, exerts very similar effects in rat and cat models during activation of sympathoexcitatory reflexes. EA reduces the sympathoexcitatory response through an opioid mechanism involving μ and δ opioid receptors in the rostral ventrolateral medulla (rVLM), an important region of cardiovascular regulation that controls sympathetic outflow (24). In addition, δ and κ opioid receptors in the spinal cord have been documented to play a role in mediating somatic nerve stimulation-induced modulation of cardiovascular reflex responses (42).

The spinal cord processes somatic and visceral reflexes as well as outputs from the central nerve system (CNS) to effector organs involved in cardiovascular reflex regulation (26). Nociceptin, a recently discovered ligand for the nociceptin/orphanin FQ peptide (NOP) receptor, is located in a number of sites in the CNS, including the rVLM and the spinal cord (1, 13, 17, 31). EA inhibits sympathoexcitatory reflex responses to gastric distension through activation of NOP receptors in the rVLM (9). Nociceptin has been shown to be located in the dorsal horn, ventral horn and intermediolateral column (IML) of the spinal cord (1, 13). However, the roles of spinal nociceptin and its neural pathway in EA-related inhibition during visceral sympathoexcitatory reflexes have not been defined. Thus, the aim of this study was to elucidate the mechanisms underlying role of spinal nociceptin in acupuncture-related regulation of cardiovascular...
sympathoexcitatory reflexes using a combined physiological and pharmacological approach. We hypothesized that nociceptin acting through NOP receptors in the thoracic spinal cord is responsible, in part, for the inhibitory influence of EA on cardiovascular visceral reflex responses.

METHODS

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committees of the University of California, Irvine and Los Angeles, CA. The study conformed to the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings”. Studies were performed on adult Sprague-Dawley rats (400-600 g). After an overnight fast (18 h), anesthesia was induced with ketamine (100 mg/kg, im) and xylazine (10 mg/kg, im). The right jugular vein was cannulated for administration of sodium bicarbonate and saline. The trachea was intubated and respiration controlled with a small animal ventilator (SAR-830/P, CWE). A 3-F pressure catheter was inserted into the right or left carotid artery (SPR-524, Millar Instrument) to monitor systemic BP. Heart rate (HR) was derived from the pulsatile BP signal. Arterial blood gases and pH were measured periodically with a blood gas analyzer (IRMA TRUPOINT, ITC) and were kept within normal physiological limits (PCO₂ 30-40 Torr and PO₂ >100 Torr) by adjusting the ventilatory rate or volume and enriching the inspired O₂ supply. Arterial pH was maintained between 7.35 and 7.43 by infusion of a solution of 8% sodium bicarbonate. Body temperature was kept between 36 and 38 °C with a heating pad.

A 3-cm (unstressed dimension) latex balloon was attached to a polyurethane tube (3-mm diameter) that was inserted into the stomach through the mouth and esophagus. A syringe was attached to the cannula to inflate and deflate the balloon with air. Distension pressures were selected to fall within the range that a rat normally experiences during ingestion of food and fluids in a single meal (2, 10, 11). Within 5-10 s of inflation, we noted an increase in systemic arterial BP. To evaluate action of
nociceptin at the spinal cord level, we inserted a tube (PE-10) into the spinal subarachnoid space at the level of T1-2 through an incision made on the atlanto-occipital membrane in the anesthetized rats. We chose intrathecal injection at T1-2 because the sympathetic preganglionic neurons that provide innervation to the heart are located in the IML of the thoracic cord at T1-3 (37). In addition, afferents from the median nerve, which were stimulated during EA, enter the spinal cord at a high thoracic level. To confirm the spinal location of nociceptin’s action in EA-related cardiovascular regulation, we performed a laminectomy to expose the high thoracic region of the spinal cord (T1) and microinjected nociceptin antagonist through a pair of glass micropipettes (30 μm tip diameter) positioned stereotaxically at the superficial lamina (I-III) of the dorsal horn or the IML. A previous study has demonstrated that microinjections of nociceptin into the IML at this level can cause a bradycardia response (7).

Seven animals were placed in a stereotaxic head frame (Kopf Instruments) with the head held rigidly by steel plugs introduced through the auditory meatus and by bars holding the upper jaw. The dorsal medulla was exposed by a midline incision in the skin, separation of the overlying muscles and a partial occipital craniotomy. A stainless electrode was inserted unilaterally 90° perpendicular to the dorsal surface, 1.7 mm lateral and 1.8 mm rostral to the calamus scriptorius at a depth of 3.2 mm to approach the rVLM (9, 44). A stimulation current (0.5 ms pulse, 10 Hz, 0.4 mA) was sufficient to produce an increase in BP (>25 mmHg). The pontamine blue dye mixed with the nociceptin antagonist was microinjected to mark the microinjection sites in the dorsal horn, IML and rVLM. The spinal cord and brain stem were removed and fixed in 4% paraformaldehyde and 20% sucrose. They were cut in 40 μm coronal sections with a freezing microtome, then mounted on slides. Sites in the brain stem where the current produced the increases in arterial BP were marked by a single electrode track. Sites were plotted on coronal sections. The areas of electrical stimulation in the rVLM and microinjections in the spinal cord were identified by the atlas of Paxinos and Watson (33).
**Experimental Protocols**

After the surgical procedures, we allowed a 30-min period of stabilization before beginning the experimental protocols. The balloon was inflated every 10 min throughout each experiment by injecting 5-10 ml of air for 30 s, a volume that induced a distension pressure of ~20 mmHg (41). The volume of air used for distension was maintained constant for each animal throughout the protocol. Ten-minute intervals between inflations prevented tachyphylaxis of the cardiovascular responses (23, 41). After the maximal cardiovascular pressor response was observed, air was withdrawn from the balloon. BP and HR responses were recorded and analyzed offline with data acquisition software Power Lab (AD Instruments). The stomach was distended repeatedly every 10 min throughout the protocol.

All experimental drugs were purchased from Sigma Aldrich (St. Louis, MO). Drugs were dissolved in normal saline at room temperature to an initial concentration of 1 mg/ml. Stock solutions were stored in a freezer and used within 2 wk, after which a fresh stock solution was prepared. On day of the experiment, appropriate serial dilutions were made to obtain desired concentrations. The concentration of naloxone, nociceptin and the nociceptin antagonist \{[N-Phe\textsuperscript{1}]-nociceptin (1-13) NH\textsubscript{2}\} was 10 nM. The volumes for intrathecal injection and microinjections into the dorsal horn and IML were 10 µl and 30 nl, respectively. The 30 nl injection was administered over a period of 10 s. The coordinates for the IML at T\textsubscript{1} were 0.8-1 mm lateral to the midline and 0.8-1 mm deep from the dorsal surface of the spinal cord. The doses and volumes were based on preliminary studies demonstrating consistent responses. We did not observe dye spread from the dorsal horn to the IML or vice versa. The distance from the injection site in the superficial lamina of the dorsal horn to the IML is approximately 0.7 mm. Nealey has not observed spread of 100 nl injectate beyond 0.5 mm from the site of injection (30).

**Gastric Distension Response to Nociceptin in the Spinal Cord**

Intrathecal NOP: In eight animals, after observing two repeatable responses to gastric distension we intrathecally injected the NOP receptor agonist nociceptin (10 µl, 10 nM)
at T_{1-2} followed by repeated gastric distension every 10 min for an additional 70 min. Vehicle control: In five rats, after two repeatable responses to gastric distension, saline (10 µl) was injected intrathecally at T_{1-2} as the vehicle control.

Opioid Antagonism in Nociceptin Response

Naloxone + NOP blockade with [N-Phe^1]-nociceptin (1-13) NH₂: Eight animals were pretreated with naloxone (10 µl, 10 nM) followed by intrathecal injection of the NOP agonist and followed by the NOP receptor antagonist, [N-Phe]-nociceptin (1-13)NH₂ (10 µl, 10 nM) (3, 4). [N-phe]nociceptin(1-13)NH₂ is the first truly selective and competitive nociceptin receptor antagonist devoid of any residual agonist activity (4).

Effect of EA on Pressor Response to Gastric Distension, Influence of Nociceptin Antagonism

EA effect: In seven rats, after observing two repeatable responses to gastric distension, we induced 30 min of EA (2 Hz, 2-4 mA, 0.5 ms duration) at P 5-6 followed by repeated gastric distension. EA + NOP blockade: Eight rats were subjected to an identical protocol with the exception that the NOP antagonist was intrathecally injected at T_{1-2} 20 min after beginning EA followed by repeated gastric distension every 10 min for an additional 50 min. EA + NOP blockade + naloxone: In seven rats, the NOP antagonist and naloxone were injected intrathecally at T_{1-2} 20 min after beginning EA followed by repeated gastric distension every 10 min for an additional 50 min.

Spinal Nociceptin System in Cardiovascular Responses to Electrical Stimulation of rVLM

rVLM stimulation + NOP: Intrathecal injection of nociceptin at T_{1-2} was performed in seven animals followed by electrical stimulation of the rVLM.

Spinal Neural Pathway of Nociceptin in EA-modulation of Cardiovascular Reflex

EA + NOP blockade in dorsal horn: In seven rats, the NOP antagonist (30 nl, 10 µM) was microinjected bilaterally into the dorsal horn at T₁ 20 min after initiation of EA followed by repeated gastric distension every 10 min. EA + NOP blockade in IML: In six
rats, the NOP antagonist was microinjected bilaterally into the IML at T1-20 min after the
start of EA followed by repeated gastric distension every 10 min.

**Statistical Analysis**

Data are presented as the means ± SE. Mean arterial pressure (MAP) at rest were
compared over time using a repeated-measures ANOVA followed post hoc by the
Tukey test. Gastric distension-responses also were assessed by a one-way repeated
measure of ANOVA, followed by the Tukey test to compare BP responses before,
during and after EA in each group. Statistical calculations were performed with
SigmaStat software (Jandel Scientific Software, San Rafael, CA). Differences were
considered significant when P < 0.05.

**RESULTS**

**Gastric Distension Response to Nociceptin in the Spinal Cord**

The gastric distension-induced pressor response of 26 ± 4 mmHg was reduced to 17 ±
3 mmHg by nociceptin administered intrathecally at T1-2, representing a 35% change.
Original blood pressure tracings are displayed in Fig.1A. Inhibition lasted for 50 min
before returning to 24 ±3 mmHg (Fig. 1B). Intrathecal injection of nociceptin did not
alter HR or resting BP before gastric distension. Intrathecal injection of saline did not
alter the pressor responses to gastric distension (n=5).

**Opioid Antagonism in Nociceptin Response**

The magnitude of the pressor response induced by gastric distension was unaltered by
intrathecal injection of naloxone at T1-2. However, in the presence of opioid receptor
blockade, spinal nociceptin promptly reduced the pressor response by 32% (Fig. 1C)
with inhibition lasting for 50 min. Intrathecal injection of the nociceptin antagonist ([N-
Phe1]-nociceptin (1-13) NH2) reversed the nociceptin-induced inhibition for 20 min.
Resting arterial BP and HR remained constant throughout this protocol.
**Effect of EA on Pressor Response to Gastric Distension, Influence of Nociceptin Antagonism**

The inhibitory effect of low-frequency EA at P 5–6 was evaluated in seven animals (Fig. 2A). Baseline BP and HR were not significantly altered during the period of the experiment. Thirty min of EA attenuated the gastric distension induced pressor reflex from 23 ± 3 to 15 ± 4 mmHg (35%), a response that persisted for 30 min after termination of EA. Original blood pressure tracings of an individual animal demonstrate the inhibitory modulation by EA of the pressor reflex as well as the effect of nociceptin antagonism during EA (Fig. 2a).

Intrathecal injection of the nociceptin antagonist at T1-2 immediately following 20 min-EA partially reversed the EA-related modulation of the visceral reflex from 15 ± 2 mmHg to 21 ± 3 mmHg (Fig. 2B), responses that were not different from those observed during the pre-EA control period but which were significantly higher than the responses during EA. A combination of nociceptin and classical opioid receptor antagonism with [N-Phe\(^1\)]-nociceptin (1-13) NH\(_2\) and naloxone completely abolished the EA inhibitory effect. Resting BP and HR were unchanged throughout this protocol (Fig. 2C).

**Spinal Nociceptin System in Cardiovascular Responses to Electrical Stimulation of rVLM**

Unilateral electrical stimulation of the rVLM elicited pressor responses averaging 24 ± 3 mmHg. Intrathecal injection of nociceptin attenuated the pressor responses by 45% to 13 ± 2 mmHg (Fig. 3A). Examination of the rat brain slices revealed that the electrical stimulation sites were within the rVLM (9, 44). The stimulation sites were confined to an area that was 2.0–3.0 mm caudal to interaural line, 1.6–2.1 mm lateral to the midline, 0.2–1.0 mm from the ventral surface, lateral to the inferior olive nucleus and the pyramidal tracts, as well as ventral and medial to the facial and retrofacial nuclei (Fig. 3B).

**Spinal Neural Pathway of Nociceptin in EA-modulation of Cardiovascular Reflex**
Bilateral microinjection of the nociceptin antagonist into either the dorsal horn or the IML at T1 partially reversed the inhibitory effect of EA on the excitatory cardiovascular reflex responses to gastric distension (Fig. 4A, B). The composite anatomical map shows the location of the microinjection sites in the dorsal horn and the IML in the high thoracic region (Fig. 4C).


DISCUSSION

In this study we investigated the role of the opiate-like peptide nociceptin in the spinal cord with respect to its contribution to EA’s inhibitory effects on cardiovascular function. We made several novel observations. First, intrathecal injection of nociceptin at T1,2 attenuated the gastric distension-induced reflex pressor responses, very similar to the influence of EA at P 5-6 on reflex sympathoexcitatory responses. Second, intrathecal injection of the NOP antagonist ([N-Phe1]-nociceptin(1–13)NH2) partially reversed the EA response. Third, pretreatment with the opioid receptor antagonist naloxone did not alter the EA-like inhibitory influence of nociceptin on the pressor reflex, while a combination of naloxone and nociceptin receptor antagonism completely abolished the EA effect. Fourth, intrathecal injection of nociceptin attenuated the pressor responses to electrical stimulation of the rVLM suggesting that nociceptin can act on sympathetic outflow. Fifth, bilateral microinjection of NOP antagonist into either the superficial lamina (I-III) of the dorsal horn or the IML of the spinal cord at T1 partially reversed the EA inhibitory effect. Taken together, these results suggest that nociceptin and the associated NOP receptor in the spinal cord contribute to the inhibitory effect of EA on the reflex autonomic responses during mechanical stimulation of the stomach, through a mechanism that is independent of the classical opioid system.

Our previous studies in experimental models and in human subjects have shown that acupuncture does not significantly alter baseline BP, but that it is capable of lowering elevated BP (21, 41-43). In this regard, reflex excitatory cardiovascular responses are significantly reduced when low-current and low frequency EA (0.3– 0.5 mA, 2 Hz) stimulation is applied at P 5–6 acupoints in rats (23, 41, 42). As such, in the present study we stimulated an abdominal visceral reflex to elevate BP. Stimulation of mechano- or chemosensitive receptors in a number of abdominal visceral organs like the stomach activates reflex responses in the cardiovascular system (27, 28). In particular, stimulation of mechanosensitive C and Aδ-fiber spinally projecting afferents in the stomach reflexly increases sympathetic tone to the heart, blood vessels and adrenal medulla (27). Thus, either passive gastric distension within physiological range
or application of capsaicin that stimulates C fibers in a cat significantly increases BP, HR, and myocardial contractility (27, 28). Distension in the rat’s stomach likewise increases BP (23, 41), and thus provides an appropriate model to study the influence of acupuncture on the cardiovascular responses to reflex activation.

Several CNS regions including the arcuate nucleus, periaqueductal gray and rVLM, serve as important nuclei that process EA’s influence on the cardiovascular system (5, 9, 25, 38, 44). More specifically, EA inhibits sympatoexcitatory reflex responses to gastric distension through activation of NOP receptors in the rVLM (9). NOP receptors are located in a number of sites in the CNS, including the rVLM and the spinal cord (1, 13, 17, 31). Nociceptin modulates sympathetic outflow at several anatomical levels. At a supraspinal level, it has been shown that bilateral injection of nociceptin into the rVLM reduces BP and HR in rats (8). Thus, nociceptin acts centrally to inhibit central sympathetic outflow consequently producing bradycardia and hypotension (18, 36). Spinally, nociceptin-like immunoreactivity has been found to be present in nerve fibers of the dorsal horn (1, 13) as well as in somata and fibers of the IML (1, 13), suggesting that nociceptin may participate in regulation of sensory as well as autonomic function.

We have found that enkephalin and dynorphin but not β-endorphin in the spinal cord plays an important role in magnetic stimulation-related modulation of cardiovascular reflex responses (42). In the present study, nociceptin receptor antagonism, even in the presence of prior blockade with naloxone, completely abolished EA modulation of reflex excitatory responses, indicating that the nociceptin system in the spinal cord plays an important role for EA-related cardiovascular regulation, independent from any action of opioids.

As an inhibitory neurotransmitter, nociceptin plays a role in regulation of cardiovascular responses. In anesthetized rats, intravenous injection of nociceptin produces a transient, dose-dependent fall in systemic BP, accompanied by a reduction in HR (14). Intracerebroventricular injection of nociceptin also triggers hypotension and bradycardia (18). Interestingly, intrathecal injection of nociceptin does not alter the resting BP and HR, but significantly attenuates the pressor reflex responses to gastric distension,
suggesting that spinal and systemically administered nociceptin likely regulate the cardiovascular system through actions on separate centers and/or pathways. Furthermore, these data also suggest that nociceptin in the spinal cord does not provide tonic modulation of blood pressure.

The spinal cord is an important integrative region of afferent and efferent pathways that participates in cardiovascular regulation. Anatomical and physiological studies indicate that the dorsal horn of the spinal cord serves as a major center for EA-induced analgesia (19, 20). Both low and high frequency EA at Zusanli (St 36) acupoint increase Fos immunoreactive neurons in the superficial laminae (I and II) in the dorsal horn of the spinal cord (19). Since nociceptin-like immunoreactivity is present in the spinal sympathetic nuclei (i.e., IML) (13), it is possible that EA also influences neurotransmission between the brain stem and the IML (13, 16). In this study, we did find that nociceptin reduced the response to rVLM-induced sympathoexcitation, indicating that nociceptin can regulate sympathetic outflow. In addition there has been a suggestion that descending pathways from the brain stem (presumably to the dorsal horn of the spinal cord) may influence segmental processing of somatic inputs during EA (15, 34, 35). Afferent stimulation can modulate sympathetic activity through inhibition of excitatory interneurons (39). In addition, somatic stimulation can elicit excitatory and inhibitory responses in both IML and dorsal horn interneurons depending on the dermatome stimulated (6). These interneurons appear to form important links in the spinal cord circuitry involved in autonomic control (12). In the present study, we observed that bilateral microinjection of the NOP antagonist in the superficial lamina (I to III) of the dorsal horn or the IML at T1, partially reversed EA modulation of the visceral excitatory cardiovascular reflex. We speculate that nociceptin serves a role in the processing of spinal cord interneuron activity in the EA response. However, spinal circuits controlling the cardiovascular visceral reflex responses during EA require further elucidation.

In conclusion, these data provide the first documentation that the endogenous nociceptinergic system in the spinal cord contributes to the inhibitory actions of EA on
the excitatory reflexes elicited by mechanical distension of the stomach. In this regard, antagonism of nociceptin’s action in the spinal cord during EA reverses the inhibitory action of EA on the pressor reflex during visceral afferent stimulation. Additionally, in the absence of EA, intrathecal injection of exogenous nociceptin at T1-2 elicits EA-like attenuation of the reflex increase in BP. Pretreatment of nociceptin with a nonselective opioid receptor antagonist, naloxone, does not alter the EA-like inhibitory influence of nociceptin on the gastric distension induced pressor reflex, suggesting that at least part of nociceptin’s actions are independent of the opioid system. Microinjection of the NOP antagonist into either the dorsal horn or the IML at T1 significantly reversed the EA responses, supporting the observation that nociceptin may act as sympathetic interneurons in EA-related cardiovascular regulation, possibly through a dorsal horn-IML pathway. These results provide new information about the spinal mechanisms underlying the influence of EA on the autonomic and cardiovascular systems.

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REFERENCES


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**Figure legends**

Fig. 1. Gastric distension response to nociceptin in the spinal cord. A: blood pressure tracings of an individual animal, represented by a, b, and c, are displayed above representative bar histograms. Small arrows on tracings indicate time of gastric distension. B: Effect of nociceptin injected intrathecally at T1-2 (long arrow) on MAP responses to gastric distension. C: Effect of naloxone and NOP receptor antagonist on nociceptin-induced attenuation of pressor responses induced by gastric distension. Baseline blood pressures before distension are indicated below each bar. Gastric distension was repeated every 10 min. Bars show increase in MAP (±SE) induced by distension of the stomach. * Significant difference after intrathecal injection of nociceptin, P < 0.05.

Fig. 2. Influence of opioid antagonism on nociceptin response. A: effect of electroacupuncture (EA) on reflex blood pressure responses. B: blood pressure responses to intrathecal injection of nociceptin receptor antagonist after onset of EA. a-d labels on the bars correspond to the tracings shown above the bars. C: blood
pressure responses to intrathecal injection of both nociceptin and opioid receptor antagonist during EA. * Significant difference compared with pre-EA. P < 0.05.

Fig. 3. Spinal nociceptin system in cardiovascular responses to electrical stimulation in the rVLM. A: Effect of intrathecal injection of nociceptin on the BP responses to electrical stimulation of the rVLM (bars). * Significant difference after intrathecal injection of nociceptin, P < 0.05. B: Composite map displaying sites of insertion of stimulation electrode (*) in the rVLM. All insertions were unilateral (side chosen randomly). Sections are 2.0–2.5 and 2.5–3.0 mm caudal to the interaural line [Paxinos and Watson, 1999]. Py, pyramidal tract; Amb, ambiguous nucleus; 7, facial nucleus; Sp5, spinal trigeminal nucleus; ION, inferior olivary nucleus.

Fig. 4. Spinal neural pathway of nociceptin in EA modulation. Microinjection of NOP antagonist in the dorsal horn (Panel A) or the IML (Panel B) at T1 following 20 min of EA, P < 0.05. Bars represent pressor responses to gastric distension. C: Composite map displaying sites of microinjections in the dorsal horn and the IML.
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A

Nociceptin antagonist (DH) (N = 7)

$\Delta$MAP (mmHg)

B

Nociceptin antagonist (IML) (N = 6)

$\Delta$MAP (mmHg)

C

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