Nociceptin in rVLM mediates electroacupuncture inhibition of cardiovascular reflex excitatory response in rats

Melissa M. Crisostomo, Peng Li, Stephanie C. Tjen-A-Looi, and John C. Longhurst. Nociceptin in rVLM mediates electroacupuncture inhibition of cardiovascular reflex excitatory response in rats. J Appl Physiol 98: 2056-2063, 2005. First published January 13, 2005; doi:10.1152/japplphysiol.01282.2004.—Electroacupuncture (EA) at Neiguan-Jianshi acupoints through an opioid mechanism inhibits the cardiovascular pressor response induced by mechanical stimulation of the stomach. Because nociceptin also may regulate cardiovascular activity through its action in the brain stem, we hypothesized that this neuropeptide modulates the pressor effect in the EA-related inhibitory effect. Blood pressure in ventilated male Sprague-Dawley rats (400–600 g) anesthetized by ketamine and α-chloralose was measured during balloon inflation of the stomach. Gastric distension with 6–8 ml of air induced consistent pressor reflexes of 26 ± 1 mmHg that could be repeated every 10 min for 100 min. When nociceptin (10 nM) was microinjected into the rostral ventrolateral medulla (rVLM), the pressor response induced by gastric distension was inhibited by 68 ± 6%. Thirty minutes of EA also decreased the reflex response by 75 ± 11%; microinjection of saline into the rVLM did not alter the inhibitory effect of EA. In contrast, microinjection of a nociceptin receptor antagonist into the rVLM promptly reversed the EA response. Pretreatment with the opioid receptor antagonist naloxone did not influence the EA-like inhibitory effect of nociceptin on the distension-induced pressor reflex (22 ± 1 to 8 ± 2 mmHg). Furthermore, a μ-opioid receptor agonist microinjected into the rVLM after microinjection of a nociceptin receptor antagonist during EA promptly reversed the nociceptin receptor antagonist-related inhibition of the EA effect. Thus, in addition to the classical opioid system, nociceptin, through opioid receptor-like-1 receptor stimulation in the rVLM, participates in the modulatory influence of EA on reflex-induced increases in blood pressure.

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RECENTLY, A NOVEL OPIATE-LIKE heptadecapeptide was identified in rat brain extracts (41, 46). The peptide, called nociceptin (41) or Orphanin FQ (46), has a high amino acid sequence homology to classical endogenous opioid peptides (i.e., endorphins, enkephalins, endomorphins, and dynorphins) and especially to dynorphin A. Furthermore, amino acid sequencing of the receptor believed to be specific for nociceptin, or opioid receptor-like-1 (ORL-1), was found to be 47–50% identical to that of the coding sequences of μ-, δ-, and κ-opioid receptors (10, 42, 46).

Nociceptin and ORL-1 receptors are reported to be abundantly distributed in neurons throughout the central nervous system (CNS), including the rostral ventrolateral medulla (rVLM), sensory trigeminal complex, raphe nuclei, locus ceruleus, periaqueductal gray, amygdala, and hypothalamic and septal regions (1, 29, 30, 42, 44, 49). These locations suggest that the nociceptinergic system may play a role in modulation of physiological activities, such as regulation of pain and autonomic outflow, much like the classical endogenous opioid system.

For centuries, acupuncture has been used by Eastern cultures to treat cardiovascular diseases such as hypertension, hypotension, arrhythmias, and conditions associated with cardiac or limb ischemia. In recent years, Western society has demonstrated a heightened interest in this treatment modality. In 1993, Eisenberg et al. (22) reported that one in three individuals obtained acupuncture or another alternative therapy. A consensus conference was convened in 1997 by the National Institutes of Health to address the efficacy and biological effects of acupuncture (42a). This conference concluded that acupuncture was useful as a complementary or alternative treatment to conventional therapy for pain, nausea, and vomiting and possibly for cardiovascular disease such as myocardial ischemia, arrhythmias, and hypertension. Additionally, the conference reported that acupuncture was being widely practiced by thousands of health practitioners for the management of pain and other health conditions. Although the medical community is beginning to recognize the value of acupuncture as a viable form of therapy, there is still much to be learned about its mechanisms of action.

Studies have begun to elucidate the mechanisms of acupuncture with regard to its therapeutic effects in cardiovascular disorders (4, 6, 7, 15, 24, 31, 34, 38, 47, 53). For example, it has been suggested that the influence of electroacupuncture (EA) on the cardiovascular system is mediated, at least partially, by the release of endogenous opioid peptides, which act as neurotransmitters that modulate the activity of premotor sympathoexcitatory cells in the brain stem medulla (15, 34, 53, 54). Our laboratory previously reported that EA modulates the reflex cardiovascular response induced by chemical and electrical stimulation of visceral afferent nerves in cats (15, 33, 34, 53, 54). In these studies, we noted that EA improves ischemic myocardial dysfunction (15, 34) by reducing demand-induced ischemia (33, 34). These responses are mediated through inhibition of autonomic reflex responses (34) specifically through the influence of EA on cardiovascular premotor sympathetic neuronal discharge in the rVLM (53, 54). In these studies, we demonstrated that the cardiovascular effects of EA were mediated by endogenous opioids and that an important site of action was the rVLM.
Nociceptin appears to regulate cardiovascular activity by influencing the peripheral nervous system (2, 13, 14) and CNS (13, 14, 17–19). Like classical opioids, nociceptin inhibits cardiovascular activity (18), decreases cardiac output and total peripheral resistance (13), and inhibits neuronal activity in the rVLM (19). It is possible that nociceptin in the CNS also contributes to the effects of EA. Nociceptin could function through the opioid system or in parallel with opioids to evoke inhibitory cardiovascular effects of EA. At present, there is no evidence to implicate the endogenous nociceptinergic system in the rVLM in the acupuncture-induced inhibition of the cardiovascular sympathoexcitatory reflexes.

In the present study, we hypothesized that, like the opioid system, nociceptin in the rVLM modulates cardiovascular reflex responses during visceral afferent stimulation. We further hypothesized that nociceptin works independently from the classical opioid system. To investigate these hypotheses, in a model of gastric distension-induced reflex cardiovascular excitatory responses, we utilized percutaneous EA and blockade and stimulation of the classical opioid system in the rVLM before and after pharmacological manipulation of the nociceptin system. A preliminary report of this work has been presented (20).

METHODS

Surgical Preparations

Experimental preparations and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Irvine, CA. The study conformed to the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” (American Physiological Society, 2002). Studies were performed on adult Sprague-Dawley male rats (400–600 g). After an 18-h overnight fast, anesthesia was induced with ketamine (100 mg/kg im) and was followed by α-chloralose (5 mg/kg iv). Additional doses of α-chloralose were given as necessary to maintain an adequate level of anesthesia, as determined by the lack of response to noxious toe pinch, a respiratory pattern that followed the respirator, as well as a stable blood pressure and heart rate (HR). The right or left jugular vein was cannulated for administration of fluids. The trachea was intubated, and the animals were artificially ventilated with a respirator (model 661, Harvard Apparatus). The right or left carotid artery was cannulated and attached to a pressure transducer (P23XL, Ohmeda) to monitor arterial blood pressure. HR was derived from the pulsatile blood pressure signal. Arterial blood gases and pH were measured periodically with a blood-gas analyzer (ABL5, Radiometer America) and were kept within normal physiological limits (\( \text{PCO}_2 \) 30–40 Torr, \( \text{PO}_2 \) > 100 Torr) by adjusting ventilatory rate or volume and enriching the inspired O\(_2\) 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 2-cm-diameter (unstressed dimension) latex balloon (Traub) was attached to a polyurethane tube (3-mm diameter) that was inserted into the stomach through the mouth and esophagus (31). The balloon was manually palpated during insertion as it passed through the esophagus into the stomach to verify positioning of the balloon inside the stomach. A syringe was attached to the cannula to inflate and deflate the balloon with air, while a manometer through a T-connection was used to monitor balloon pressure. Transmural pressure was determined by measuring the pressure required to inflate the balloon with the various volumes of air before it was inserted into the stomach. The balloon then was inflated inside the stomach, and pressure was recorded.

Experimental Procedures

Transmural pressure was calculated as the difference in pressure of the balloon inside and outside the stomach at corresponding volumes. Volumes ranging between 6 and 8 ml of air were injected into the stomach of each animal, which generated transmural pressures of 11 ± 1 mmHg. Injection volumes were selected to achieve transmural pressures ≤ 20 mmHg, a value that has been reported by others to be below the pain threshold (4) in rats (52). These distension pressures fall within the range that a rat normally experiences during ingestion of food and fluids in a single meal (21).

Animals were placed in a stereotaxic head frame, and their heads were positioned such that the floor of the fourth ventricle was horizontal. A partial occipital craniotomy was performed to expose the dorsal medulla. A three-barrel pipette (A-M Systems, Everett, WA; total outer diameter of the multibarrel tip, 45–60 μm) was inserted unilaterally (side chosen randomly) into the medulla at an angle of 90° relative to the dorsal surface of the medulla, 1.5 mm lateral from the midline and 1.8 mm rostral to the obex, and was advanced 3.2 mm from the dorsal surface toward the ventral surface. The pipette was positioned relative to the obex (interaural –4.3 mm) using landmarks, as depicted in the atlas of Paxinos and Watson (45). These coordinates provide access to a region in the rVLM that has been found by others to contain premotor sympathoexcitatory cells (25). An injection cannula and microsyringe (Hamilton) fixed to one of the barrels of a pipette were used to administer a small dose of α-homocysteic acid (DLH, 3–4 nM, 100 nl) to identify cardiovascular pressor sites at the beginning of each experiment. Each site initially was confirmed by observing a small increase in blood pressure (5–10 mmHg) without damaging the neuron (39). In control experiments, one of the micropipette barrels was filled with saline, whereas the remaining two barrels were filled with either DLH or dye.

At the end of each experiment following administration of drug into the medulla, the injection site was marked with an injection of 100 nl of Chicago Sky Blue dye (5% in 0.5 M sodium acetate). Thereafter, rats were euthanized with intravenous saturated KCl under deep anesthesia. The stomach then was exposed to verify the location of the balloon. Only animals in which the balloon was observed to be fully within the stomach were used for data analysis. The brain stem was removed and fixed in 4% paraformaldehyde and 20% sucrose for at least 4 days. Frozen 60-μm coronal sections were cut with a cryostat microtome (CM 1850, Leica) and then stained with 1% crystal violet.

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. The stock solutions were stored in a freezer and used within 2 wk, after which a fresh stock solution was prepared. On the day of the experiment, appropriate serial dilutions were made to obtain desired concentrations. Concentrations of the DLH and μ-opioid agonist \{[\text{D-Ala}^2,\text{N-Me-Phe}^4,\text{Gly}^5,\text{ol}-\text{enkephalin acetate salt (DAGO)}]\} were 3–4 and 6–12 nM, respectively. The concentration of the final solution of nociceptin, naloxone, and nociceptin receptor antagonist was 10 nM for each 100-nl microinjection into the rVLM.

Experimental Protocols

Following the surgical procedure, we allowed a 30- to 60-min period of stabilization before beginning the experimental protocol. Gastric distension was induced by inflating the balloon slowly within 10 s and was held for an additional 10 s, and then the injected air was slowly withdrawn from the balloon. We typically noted an increase in...
systemic arterial blood pressure within 5–10 s following inflation. After the maximal cardiovascular pressor response, air was withdrawn from the balloon. Ten-minute recovery intervals prevented attenuation of the cardiovascular responses (31).

Protocol 1A: responses to repeated gastric distension. In the time control group, rats were subjected to 10 repeated periods of gastric distension without EA. The total time over which the stomach was repeatedly stimulated lasted 100 min.

Protocol 1B: response to nociceptin antagonist in rVLM. We examined the effect of nociceptin antagonist on the reflex pressor responses to gastric distension. Nociceptin was delivered unilaterally into the rVLM, in the absence of EA, following by an 80-min period during which the reflex responses to gastric distension were examined repeatedly. Thus the stomach was inflated at 10-min intervals over a period of 100 min, two times before, and eight times after microinjection of nociceptin.

Protocol 2A: influence of naloxone on nociceptin response. Similar to protocol 1, animals were instrumented to allow microinjection into the rVLM. After two repeatable control responses to gastric distension, 0.1 μl nociceptin (10 nM) was microinjected into the rVLM, in the absence of EA, followed by an 80-min period during which the reflex responses to gastric distension were examined repeatedly. Thus the stomach was inflated at 10-min intervals over a period of 100 min, two times before, and eight times after microinjection of nociceptin.

Protocol 2B: influence of naloxone on nociceptin response. Similar to protocol 1, animals were instrumented to allow microinjection into the rVLM. After two repeatable control responses to gastric distension, 0.1 μl naloxone (10 nM, 0.1 μl), which we have shown blocks opioid receptors (31), was microinjected unilaterally into the rVLM. After 5 min, the response to gastric distension was recorded. Five minutes after gastric distension, nociceptin was microinjected at the same site in the rVLM, followed by a 70-min period during which the reflex responses to repeated gastric distension were recorded. The control group in experiment 1B served as control for this protocol.

Protocol 3A: influence of EA on cardiovascular response to gastric distension, microinjection of vehicle in rVLM. After recording two repeatable pre-EA control responses to gastric distension, percutaneous EA (1–2 mA, 2 Hz, 0.5-ms duration) was performed bilaterally with 2-gauge stainless steel acupuncture needles (Suzhou Medical Appliance) at the Neiguan-Jianshi acupoints (over median nerve above the paw, pericardial meridian, P 5–6) for 30 min in rats (16, 35). Needles were inserted perpendicularly to a depth of 1–3 mm. Correct positioning of the needles at the acupoint was confirmed by observing slight repetitive paw twitches during stimulation, indicating stimulation of motor fibers in the median nerve (15, 31, 33). Twenty-five minutes after beginning EA, a saline injection, which functioned as a control for the effects of microinjection, was administered unilaterally into the rVLM. During EA and for 50 min after its termination, responses to gastric distension were recorded. The 50-min period after EA was found to be adequate for recovery from the inhibitory effects of EA on the reflex pressor response. Responses to gastric distension were induced at 10-min intervals for a total of 100 min, including two before, three during, and five after EA.

Protocol 3B: effect of nociceptin receptor antagonists on EA response. The ORL-1 receptor antagonists [Phe’-γ-(CH2-NH-Gly)2]-nociceptin(1–13)-NH2 (Sigma), [N-Phe’]-nociceptin(1–13)-NH2 (Sigma) (10 nM, 0.1 μl), or an equal volume of saline was microinjected unilaterally into the rVLM. At the time of commencement of the study, only [Phe’-γ-(CH2-NH-Gly)2]-nociceptin(1–13)-NH2 was available commercially. When the more potent antagonist [N-Phe’]-nociceptin(1-13)NH2 became available and its activity was verified to lack agonist effects (12), we incorporated [N-Phe’]-nociceptin(1-13)NH2 into this protocol. Because we observed similar responses to both antagonists, we combined these data in this study. Two reproducible pre-EA control values were obtained followed by 30 min of EA at the Neiguan-Jianshi acupoints. Twenty-five minutes after beginning EA, the nociceptin antagonist was administered unilaterally into the rVLM. Distension-response measurements were taken every 10 min for a total of 130 min, including two before, three during, and eight after EA.

Protocol 3C: effect of μ-opioid agonist on nociceptin antagonist response during EA. The μ-opioid agonist DAGO was diluted to a concentration of 6–12 nM (51). Similar to protocols 1B, 2A, and 3B, animals were instrumented for microinjection into the rVLM. Two reproducible pre-EA control values were obtained followed by 30 min of EA at the Neiguan-Jianshi acupoints. Twenty-five minutes after the onset of EA, the nociceptin antagonist [N-Phe’]-nociceptin(1-13)NH2 was administered unilaterally into the rVLM. After 5 min, the response to gastric distension was recorded. Five minutes later, DAGO was microinjected into the same side of the rVLM, followed by repeated gastric distensions during a 70-min recovery period. Thus the stomach was inflated at 10-min intervals over a period of 120 min, four times before and one time after microinjection of the nociceptin antagonist, and seven times after microinjection of DAGO.

Statistical Analysis

Data are presented as means ± SE. Change in mean arterial pressures of distension responses before, during, and after EA and after delivery of saline and experimental drugs were compared by one-way repeated-measures analysis of variance and post hoc by the Student-Newman-Keuls test. All statistical analyses were performed with the software package Sigma Stat (Jandel Scientific). The 0.05 probability level was used to determine statistically significant differences.

RESULTS

Responses to Repeated Gastric Distension

Ten repeated gastric distensions caused consistent pressor responses over a 100-min period in six rats (Fig. 1A). In the absence of EA, the pressor response was constant, averaging 26 ± 1 mmHg during the duration of the protocol.

Response to Nociceptin Antagonist in rVLM

Microinjection of a nociceptin antagonist did not alter the pressor responses in four rats (Fig. 1B), indicating that 100 nl of volume injection into the rVLM do not alter the reflexes, and the reflex response is not mediated by nociceptin in the rVLM.

Gastric Distension Response to Nociceptin in rVLM

The gastric distension-induced pressor response of 19 ± 3 mmHg was reduced to 6 ± 2 mmHg by nociceptin administered unilaterally into the rVLM in six rats, representing a 68% change. Original blood pressure tracings are displayed in Fig. 2A. Inhibition lasted for 60 min before returning to 15 ± 2 mmHg, 80 min after microinjection of nociceptin (Fig. 2A). Injection of nociceptin did not alter HR or resting blood pressure before gastric distension.

Influence of Opioid Antagonism on Nociceptin Response

The magnitude of the pressor responses induced by gastric distension was unaltered by naloxone microinjected unilaterally into the rVLM. However, in the presence of opioid receptor blockade, nociceptin in the rVLM promptly reduced the pressor response by 64% to 8 ± 2 mmHg (Fig. 2B) in seven animals. Inhibition lasted for 40 min before returning to 22 ± 1 mmHg 60 min after nociceptin. Injection of nociceptin did not change the HR responses or baseline blood pressure before gastric distension. Resting arterial pressure and HR remained constant throughout this protocol.
Effect of EA on Distension of the Stomach, Influence of Saline Microinjected in rVLM

The inhibitory effect of low-frequency EA at P 5–6 was evaluated in five animals (Fig. 3A). Although the baseline blood pressures showed some fluctuation, blood pressures before the pressor responses were not significantly different. HR also was not significantly altered during the several hours of the experiment. Before EA, the pressor responses were similar to the time control groups, averaging 22 ± 5.3 and 20 ± 4.4 mmHg, respectively. After 30 min of EA, the pressor reflex during distension of the stomach was reduced to 5 ± 4 mmHg (75%), a response that persisted for 50 min after termination of EA. Microinjection of saline into the rVLM did not alter the inhibitory effect of EA.

Influence of Nociceptin Antagonist on EA Response

EA at P 5–6 inhibited the reflex response to gastric distension from 21 ± 1 to 7 ± 1 mmHg in this group of five rats. Unilateral injection of a nociceptin antagonist into the rVLM promptly reversed the pressor response to 18 ± 1 mmHg (Fig. 3C). Subsequent administration of the µ-opioid agonist DAGO at the same site in the rVLM neutralized the antagonist’s effects, noted by a prompt return of the reduced pressor response of 7 ± 1 mmHg. This inhibition lasted 20 min, increasing to 12 ± 2 mmHg after 30 min. Injection of DAGO did not change the HR responses during gastric distension. Resting mean arterial pressure and HR were unchanged throughout this protocol.

Response of Nociceptin Antagonism to µ-Opoid Agonist During Effect of EA

EA inhibited the reflex response to gastric distension from 21 ± 1 to 7 ± 1 mmHg in this group of five rats. Unilateral injection of a nociceptin antagonist into the rVLM promptly reversed the pressor response to 18 ± 1 mmHg (Fig. 3C). Subsequent administration of the µ-opioid agonist DAGO at the same site in the rVLM neutralized the antagonist’s effects, noted by a prompt return of the reduced pressor response of 7 ± 1 mmHg. This inhibition lasted 20 min, increasing to 12 ± 2 mmHg after 30 min. Injection of DAGO did not change the HR responses during gastric distension. Resting mean arterial pressure and HR were unchanged throughout this protocol.

Resting blood pressure and HR remained constant throughout the protocol.

Fig. 2. A: increase in blood pressure in response to gastric distension and microinjection of nociceptin. B: mean reflex blood pressure responses to nociceptin (10 nM, 0.1 µl) microinjected unilaterally into rVLM in the absence of electroacupuncture (EA) in 6 rats. C: effect of opioid antagonist on nociceptin response in rVLM. Naloxone (opiod antagonist, n = 7) was microinjected unilaterally into the rVLM 10 min before nociceptin. Baseline blood pressures before distension are indicated below each bar. Bars show increase in MAP (±SE) induced by distension of the stomach. a, b, and c in the bars (B) correspond with raw data (A). *Significant difference after microinjection of nociceptin, P < 0.05.
Location of Injection Sites

Examination of the rat brain slices verified that all injections located within the rVLM had significantly influenced the reflex responses to gastric distension, whereas five injections outside the rVLM (Fig. 4) did not alter the reflex cardiovascular responses. All effective sites for each of the protocols were confined to an area 1.00–2.50 mm rostral to the obex (interaural 4.3 mm) and the hypoglossal rootlets, 2.50–3.3 mm to the right or left of the midline, 0.1–1.7 mm from the ventral surface, lateral to the nucleus inferior olive and pyramidal tract, and ventral and medial to the facial and retrofacial nuclei and includes the medial and caudal parts of nucleus paragigantocellularis (Fig. 4). These sites are located within the rVLM, as defined previously by others (8, 25, 26, 28, 40).

DISCUSSION

The inhibitory effect of EA on the visceral cardiovascular sympathoexcitatory pressor reflexes has been well characterized (15, 31, 34, 54). Several studies have observed differential cardiovascular responses to gastric distension. Our laboratory has developed a rodent model that yields consistent pressor responses (31). The present study has used this model to assess the role of the novel opiate-like peptide nociceptin in the rVLM and its contribution to EA’s inhibitory effects on the cardio-

![Graph A](image)

**Fig. 3.** A: gastric distension responses before, during, and after 30 min of EA (1–2 mA, 2 Hz) at the Neiguan-Jianshi acupoints (pericardial meridian, P 5–6) in 5 rats with control saline injection. B: blood pressure responses (means ± SE) to unilateral microinjection of nociceptin receptor antagonist (10 nM, 0.1 ml) into rVLM 25 min after onset of EA (1–2 mA, 2 Hz at P 5–6 acupoints) in 7 rats. a-e in the bars (Bb) correspond with raw data (Ba). C: stimulation of µ-opioid receptors following blockade of nociceptin receptors during EA. Nociceptin antagonist was microinjected unilaterally into the rVLM 10 min before microinjection of [d-Ala², N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAGO) (µ-opioid agonist, 6–12 nM) in 5 rats 25 min after onset of EA. Gastric distension was repeated every 10 min. Values below each bar represent baseline blood pressures before distension. *Significant difference compared with pre-EA, P < 0.05. **Significant difference after microinjection with DAGO, P < 0.05.
vascular system. Microinjection of either nociceptin receptor antagonist \([N\text{-Phe}^1]\text{nociceptin}(1–13)\text{NH}_2\) or \([\text{Phe}^1\cdot\Psi(\text{CH}_2\text{-NH})\cdot\text{Gly}^2]\text{nociceptin}(1–13)\text{NH}_2\) into the rVLM markedly reduced the EA-related response. As a surrogate for EA, injection of nociceptin into this same region of the brain stem also inhibited the reflex pressor response caused by mechanical distension of the stomach. Pretreatment with the nonselective opioid receptor antagonist naloxone in the absence of EA did not alter the gastric distension-induced pressor response, nor did it alter the inhibitory effect of nociceptin in the rVLM on the response to gastric distension. Lastly, blockade of the EA-induced response by a nociceptin receptor antagonist did not prevent the inhibitory response to a specific \(\mu\)-opioid receptor agonist DAGO. Taken together, these results suggest that nociceptin and its associated ORL-1 receptor in the rVLM contribute to the inhibitory effect of EA on the reflex autonomic responses during mechanical stimulation of the stomach, in a manner that is independent of the classical opioid system.

We began this study using \([\text{Phe}^1\cdot\Psi(\text{CH}_2\text{-NH})\cdot\text{Gly}^2]\text{nociceptin}(1–13)\text{NH}_2\) as the antagonist in the nociceptin antagonist protocols (\textit{protocols 1B} and 3\textit{B}), as this was the only inhibitor that was available commercially. It later became apparent that this inhibitor exhibited both antagonist and partial agonist behaviors (12). When the new antagonist \([N\text{-Phe}^1]\text{nociceptin}(1–13)\text{NH}_2\) became available (12), we incorporated this inhibitor into \textit{protocols 1B} and 3\textit{B}. In 11 animals, both \([\text{Phe}^1\cdot\Psi(\text{CH}_2\text{-NH})\cdot\text{Gly}^2]\text{nociceptin}(1–13)\text{NH}_2 (n = 5) and the newer antagonist \([N\text{-Phe}^1]\text{nociceptin}(1–13)\text{NH}_2 (n = 6) exhibited similar antagonistic activity when they were microinjected locally into the rVLM during pressor reflexes and EA. Similarly, \textit{protocol 3C}, utilizing only the newer antagonist, caused similar degrees of inhibition of the EA-related cardiovascular response. Thus the two protocols reinforce each other and suggest that at least part of the EA-induced cardiovascular inhibitory influence in the rVLM is mediated through nociceptin.

Previous investigations have been limited by the absence of selective pharmacological antagonists to nociceptin. The fact that two structurally dissimilar selective nociceptin receptor antagonists, \([N\text{-Phe}^1]\text{nociceptin}(1–13)\text{NH}_2 and \([\text{Phe}^1\cdot\Psi(\text{CH}_2\text{-NH})\cdot\text{Gly}^2]\text{nociceptin}(1–13)\text{NH}_2\), produced similar inhibition of the EA-related cardiovascular response strongly suggests that nociceptin plays an important role in the EA-cardiovascular system. Furthermore, demonstration in the present investigation that the endogenous ligand nociceptin caused inhibitory responses much like acupuncture supports the hypothesis that this system inhibits sympathetic outflow to the cardiovascular system.

The inhibitory effect of EA on the cardiovascular system likely is the result of excitation of group III and possibly group IV somatic afferent nerve fibers beneath the acupoint (33, 37). Stimulation of these afferents can activate inhibitory systems in the brain, resulting in the release of endogenous neurotransmitters such as \(\gamma\)-amino-\(\eta\)-butyric acid, serotonin, and endogenous opioids (32). These neurotransmitters, along with nociceptin, seem to function as neuromodulators by inhibiting areas that regulate sympathetic neuronal activity like the rostral ventrolateral medulla, a key integrative center responsible, in part, for maintaining blood pressure and cardiovascular function (9, 50).

Several regions in the CNS involved in the sympathoinhibitory effect of EA have been reported, including the nucleus accumbens in the hypothalamus, the ventral periaqueductal gray, and the nucleus raphe obscurus with a projection to the rVLM (32). Furthermore, Liu et al. (36) reported that \(c\text{-fos}\) expression in the nucleus tractus solitarius during EA may involve this nucleus in the effects of acupuncture. In situ hybridization and immunocytochemistry have been used to localize nociceptin receptors and the peptide in the sensory trigeminal complex, raphe nuclei, locus coeruleus, periaqueductal gray, amygdala, and hypothalamic and septal regions (49), as well as abundant distribution in the rVLM (2, 43). Location of the nociceptinergic system in several areas involved in central regulation of cardiovascular function suggest that it may participate in the EA-cardiovascular responses not only in the rVLM but in other regions as well.

Many reports describe the wide range of nociceptin’s cardiovascular and nociceptive effects, varying from inhibitory (17, 18) to analgesic (48) to excitatory (3) and hyperalgesic (11). Like the endogenous opioid peptides, the global action of nociceptin is dependent on the distribution of its receptors, as well as the nature of the cell that has been activated. At the
cellular level, nociceptin’s actions are generally inhibitory (27). However, Feuerstein and Fadden (23) demonstrated that microinjection of an opioid peptide agonist into two hypothalamic nuclei located only 1 μm apart led to opposite effects on arterial blood pressure in the rat. Such differences in cellular response could potentially account for the previous disparate reports characterizing nociceptin’s effects. More investigation will be required to determine whether these mechanisms account for nociceptin’s varied actions on the cardiovascular or pain systems. However, the present study provides strong evidence that nociceptin in the rVLM inhibits reflex-induced sympathoexcitatory cardiovascular responses and serves as one of the mechanisms of EA-related inhibition of excitatory pressor reflexes.

One potential limitation of this study is that the gastric distension responses in the time control group were slightly larger than the gastric distension responses observed in protocols 1B, 2A, 2B, 3A, 3B, and 3C. We suggest that this observation likely was due to the less invasive surgery in animals in the time control group compared with animals in the microinjection studies. However, it is important to note that, before EA or microinjection studies, control gastric distension responses were consistent, and all of the interventions were reversible. Therefore, we do not believe that differences in the control responses vs. those in the microinjection studies affect our conclusions.

In conclusion, these data provide the first documentation that the endogenous nociceptinergic system in the rVLM 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 rVLM during EA reversed the inhibitory actions of EA on the pressor reflex during visceral afferent stimulation. Additionally, in the absence of EA, exogenous nociceptin microinjected into the rVLM elicited EA-like attenuation of the reflex increase in blood pressure. Pretreatment of nociceptin with a nonselective opioid receptor antagonist, naloxone, did 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. Also, microinjection of a selective μ-opioid receptor agonist immediately after microinjection of a nociceptin receptor antagonist during EA rapidly reversed the nociceptin receptor antagonist-related inhibition of the EA response, indicating that activation of the opioid system during EA can function independently from the nociceptinergic system. Taken together, these data suggest that nociceptin, similar to but separate from the classical opioid system, functions as an important neuromodulator of visceral reflex cardiovascular stimulation during EA-related somatic afferent activation.

GRANTS

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REFERENCES


