EFFECT OF ELECTROACUPUNCTURE ON PRESSOR REFLEX DURING GASTRIC DISTENTION

Peng Li, Kasra Rowshan, Melissa Crisostomo, Stephanie C. Tjen-A-Looi, and John C. Longhurst

Department of Internal Medicine
College of Medicine
University of California, Irvine
Irvine, CA 92697-4075

Index words: rostral ventrolateral medulla, Neiguan acupoint, Zusanli acupoint, Naloxone, rats

Corresponding author: Peng Li, M.D.
Medical Science 1 C235
College of Medicine
University of California, Irvine
Irvine, CA 92697-4075
Tel: 949-824-6123
Fax: 949-824-2200
E-mail: pengli@uci.edu

Copyright 2002 by the American Physiological Society.
The effect of Electroacupuncture (EA) on the reflex cardiovascular response induced by mechanical distension of the stomach was studied in ventilated male Sprague-Dawley rats anesthetized by ketamine and α-chloralose. Repeated balloon inflation of the stomach to produce 20 mmHg tension on the gastric wall induced a consistent rise in mean arterial pressure (MAP), while heart rate (372±22 beats/min) was unchanged. This response was reversed by transection of the splanchnic nerves. Bilateral application of EA (1-2 mA, 2 Hz) at Neiguan-Jianshi acupoints (Pe 5-6) over the median nerve for 30 min, significantly decreased the pressor response from 33±6 to 18 ±4 mmHg (n=7, P<0.05). This effect began after 10 min of EA and continued for 40 min after termination of EA. EA at Zusanli-Shangquxu acupoints (St 36-37), over the deep peroneal nerve, similarly inhibited the pressor response. The effect lasted for 10 min after EA was stopped (n=6, P<0.05), while EA at Guangming-Xuanzhong acupoints (GB 37-39) over the superficial peroneal nerve did not inhibit the pressor response. Naloxone injected intravenously (n=6) immediately after termination of EA or administered by microinjection into the rostral ventrolateral medulla (rVLM) 25 min after initiation of EA (n=6) reversed the inhibition by EA, suggesting an opiate mechanism, including the rVLM, was involved.

INTRODUCTION

Stimulation of sensory nerves in a number of regions including chemoreceptors and mechanoreceptors in the carotid arteries and aortic arch, heart and gastrointestinal regions activates reflex responses in the cardiovascular system (7, 32, 33). For example, stimulation of mechano- or chemo-sensitive receptors in the stomach activates reflex responses in the cardiovascular system (32,
Thus, passive distension of a cat's stomach, using pressures within physiological range, or application of capsaicin, a strong stimulus of C fibers, significantly increases blood pressure, heart rate, and myocardial contractility. The afferent pathways mainly follow splanchnic nerves and, to a lesser extent, the vagus. The efferent pathways of this reflex include cardiac and visceral sympathetic outflow (32, 33, 34). The neural centers for integrating this reflex have not been well studied. However, the rostral ventrolateral medulla (rVLM) is one region that integrates sympathetic outflow during cardiovascular reflexes (19, 49), including input from the splanchnic nerve.

Eating also is associated with marked rises in heart rate, cardiac output and oxygen consumption (11). These increases in heart rate and systemic blood pressure can contribute to myocardial ischemia and angina in susceptible patients (42). Thus, augmentation of myocardial oxygen demand during and after ingestion of food can lead to postprandial angina in patients with coronary artery disease (11, 33, 40).

Acupuncture, and its more potent alternative electroacupuncture (EA), has been used for centuries in traditional Chinese medicine to treat a variety of diseases and disorders (31) including hypertension, angina pectoris, arrhythmias, and myocardial infarction (4, 5, 6, 9, 14, 41, 50). For example, it has been shown that three 30 min sessions of acupuncture each week reduce the number of anginal attacks compared to placebo and increase the threshold for angina pectoris during exercise (41). In addition, administration of EA for 20 min each day for three weeks increases the maximal rate-pressure product during exercise in patients with severe angina (4). These studies suggest that EA perhaps can be used to reduce the incidence of postprandial angina and myocardial ischemia.

Two acupoints, the Neiguan and Zusanli, located over the median and deep peroneal nerves, respectively, have been used by practitioners for treating a number of cardiovascular conditions (9,
Support for these acupoints also is present in experimental studies. For example, stimulation of the deep peroneal nerve below the Zusanli (St 36) acupoint or median nerve underneath the Neiguan acupoint (Pe 6) reduces the frequency of ventricular extrasystoles induced by stimulation of the hypothalamus (10, 27, 54). However, the relative efficacy of these two acupoints with regard to their effects on the cardiovascular system is not known. Some articles involving EA-like somatic nerve stimulation show a prolonged effect lasting up to 5-12 hrs (35, 52) while others involving the median/deep peroneal nerve show a much briefer response lasting only 30-40 min (8, 10, 24, 27). Our previous data suggest that EA produces a similar response as whole (median) nerve stimulation (8, 10, 27). Thus, additional investigation concerning point specificity and duration of effect using carefully controlled studies is required.

To study EA, we herein describe a model of gastric-distension in rats designed to reflexly activate the cardiovascular system. Mechanical distension of the rat stomach, within a range of volumes occurring during normal ingestion of fluid, provides a physiological model of stress that reversibly increases sympathetic tone and the corresponding pressor response.

We hypothesized that 30 min of low frequency EA similar to that used to treat cardiovascular disease (8, 27) would result in prolonged opioid-mediated inhibition of the cardiovascular pressor reflexes induced by gastric distension. Secondly, we proposed that variability in response to acupuncture at two different acupoints would be present (ie. point specificity).

**Method**
**Surgical Preparation**

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of the University of California, Irvine, CA. The study conformed to the American Physiological Society guidelines and principles for research involving animals (1). Studies were performed on adult Sprague-Dawley male rats (400-600 g). After an overnight fast (18 hrs), anesthesia was induced with ketamine (100 mg/kg i.m.) and was maintained with α-chloralose (5 mg/kg i.v.). Additional doses of α-chloralose were given as necessary to maintain an adequate level of anesthesia, as determined by the lack of response to toe pinch and the ability to artificially maintain a consistent respiratory rate. The right jugular vein was cannulated for administration of bicarbonate and the opioid antagonist, naloxone. The trachea was intubated and respiration was monitored with a ventilator (Model 661, Harvard Apparatus). The right or left carotid artery was cannulated and attached to a pressure transducer (P23XL, Ohmeda) to monitor systemic blood pressure. Heart rate 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 (PCO₂ 30 - 40 mmHg, and PO₂ > 100 mmHg) by adjusting ventilatory rate or volume and enriching the inspired O₂ supply. Arterial pH was maintained between 7.35 - 7.43 by infusion of a solution of 8% sodium bicarbonate. Body temperature was kept between 36°C and 38°C with a heating pad.

A 2 cm diameter (unstressed dimension) latex balloon (50) was attached to a polyurethane tube (3 mm diameter) that was inserted into the stomach through the mouth and esophagus. We manually palpated the balloon during insertion as it passed through the esophagus and into 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 intragastric pressure. Transmural gastric pressure was
determined by calculating the difference between the balloon pressure inflated within and outside the stomach with the same volume of air. This represented the pressure exerted on the walls of the stomach. 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, 3, 11, 12). Within 30 sec of inflation, we noted an increase in systemic arterial blood pressure. In the single occasion when the balloon was not in the stomach but remained in the esophagus, the pressor response was much larger; we eliminated this animal from consideration.

**Experimental Procedure**

After the surgical procedure, we allowed a thirty minutes stabilization period before beginning the experimental protocol. The balloon was inflated every 10 min throughout each experiment by injecting 15 ml of air within 10 sec, a volume that induced a distension pressure of approximately 20 mmHg (2, 12). Ten min intervals prevented tachyphylaxis of the cardiovascular responses. After the maximal cardiovascular pressor response, air was withdrawn from the balloon. Typically, the pressor response was observed within 5-10 sec following inflation of the balloon. After the completion of each experiment, rats were euthanized with intravenous 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 within the stomach were used for data analysis.

**Experimental Protocols**

_Gastric distension before and after splanchnic nerve transection (Protocol 1)_ To verify that gastric distension induced a reflex cardiovascular pressor response, four rats were subjected to gastric distension before and after transection of the splanchnic nerves. After completion of the surgical preparation and prior to a 30 min stabilization period, small incisions were made below each costal margin to isolate the splanchnic nerves. Once inside the peritoneal cavity, the nerves were
identified using the kidney, adrenal gland and respiratory diaphragm as landmarks. The splanchnic nerves were isolated by wrapping a 4.0-suture around each nerve.

Following recordings of two reproducible control responses to gastric distension, the splanchnic nerves were severed, and responses to the distension of the stomach were recorded. Then, intramuscular injection of 40 µg of capsaicin into the thigh was used to determine the animal’s responsiveness. Mean arterial pressure (MAP) and heart rate (HR) were recorded during the procedure.

**EA at Neiguan-Jianshi, Zusanli-Shangquxu and Guangming-Xuanzhong (Protocol 2)** After recording three repeatable pre-EA control responses to gastric distension, percutaneous EA (1-2 mA, 2 Hz, 0.5 ms duration) was performed bilaterally with 32g stainless steel acupuncture needles (Suzhou Medical Appliance, China) at either the Neiguan-Jianshi (over median nerve above the paw, pericardial meridian, Pe 5-6) or Zusanli-Shangquxu (over deep peroneal nerve below the knee, stomach meridian, St 36-37) acupoints for 30 min in 13 rats (10, 22, 29). Needles were inserted perpendicularly to a depth of 3-4 mm. Correct positioning of the needles at both acupoints was confirmed by observing slight repetitive paw twitches during stimulation (8, 27, 28). The Guangming-Xuanzhong (over superficial peroneal nerve above the lateral side of ankle, gallbladder meridian, GB 37-39) acupoints were used as controls. Needle insertion and stimulation parameters were the same as with the Neiguan-Jianshi and Zusanli-Shangquxu acupoints. Placement of the needles in these control acupoints was confirmed by noticing a slight twitch of the feet. During EA and for 50 min after its termination, responses to gastric distension were recorded. The 50 min period following 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 90-100 min, including three before, three during and four or five after EA.

In the time control group, eight rats were subjected to nine repeated periods of gastric distension without EA, each distention lasting 30 sec and repeated after 10 min while the reflex blood pressure responses were monitored. The total time over which the stomach was repeatedly stimulated was 90 min. Acupuncture needles were not inserted, since even simple insertion of EA needles can cause acupuncture-like effects (47).

**EA influence on responses to graded changes in intragastric pressure (Protocol 3)** The distension-induced pressor response was evaluated during increases in gastric volume using random increments of 5 to 25 ml in six rats, before and after 30 min of EA at the Neiguan-Jianshi acupoints. Evaluation of the response after EA commenced immediately upon termination of EA. The entire repetitive stimulation-response process lasted approximately 25 min, which was within the time frame of the effect of EA at Neiguan-Jianshi acupoints.

**Intravenous and rVLM naloxone on EA response (Protocols 4 and 5)** Naloxone (Sigma) was diluted to a concentration of 4 mg/ml in 0.9% NaCl, and its dose is 4 mg/kg. Similar to Protocol 2, blood pressure responses before EA during three separate distensions in six rats were recorded followed by 30 min of EA at the Neiguan-Jianshi acupoints. Immediately after termination of EA, 4 mg/kg of naloxone was administered intravenously (Protocol 4), followed by a 50 min recovery period. Thus, the stomach was inflated at 10 min intervals for a total of 100 min, three times before, three times during and five times after EA.

In Protocol 5, after the general preoperative surgery, animals were placed in a stereotaxic head frame, and the head of the animal was 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 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, 1.8 mm rostral to the obex, and advanced 3.2 mm from the dorsal toward the ventral surface (39). These coordinates provide access to a region in the rostral ventral lateral medulla (rVLM) that has been found by others to contain premotor sympathoexcitatory cells (19). An injection cannula and microsyringe (Hamilton) fixed to a pipette were used to administer 100 nl of naloxone (4 mg/ml) or 0.9% saline as the vehicle control into the rVLM. Two reproducible pre-EA control values were obtained followed by 30 min of EA at the Neiguan-Jianshi acupoints. Twenty-five min after beginning of EA, naloxone (n=6) or saline (n=6) was administered unilaterally into the rVLM. Distension-response measurements were taken every 10 min for a total of 100 min, including two before, three during and six following EA. At the end of the experiment the injection site was marked with microinjection of Chicago Blue dye (100 nl). Animals were euthanized with saturated KCL solution injected into the circulation under deep anesthesia. The brain stem was removed and fixed in 4% paraformaldehyde and 20% surcose for at least 48 hr. Frozen 60 µm coronal sections were cut with a cryostat microtome (International Equipment, Minotome) and stained with 1% neutral red in acetate buffer with 2% acetic acid, pH 4.8. The sites of microinjection were identified according to the atlas of Paxinos and Watson (39). Diffusion distances for dye spots ranged between 80 and 100 m in diameter.
**Statistical Analysis**

Means and standard errors of mean blood pressure and heart rate at rest were compared over time using a repeated-measures analysis of variance (ANOVA) followed by the Tukey Test to examine for nonrandom variation. In the distension-response protocol, comparisons by ANOVA were made between blood pressure responses before and after EA at each increment in gastric volume. The 0.05 probability level was chosen to determine statistical significance.

**RESULTS**

**Denervation studies (Protocol 1)** Afferent pathways activated during mechanical stimulation of the stomach are located in the splanchnic nerves (33, 34). Changes in MAP during mechanical distension before and after bilateral splanchnic nerve transection are shown in Fig. 1 (n=4). The MAP of 24 ± 5 mmHg was significantly reduced to 1 ± 6 mmHg by denervation. Baseline blood pressure was not altered by this maneuver. Furthermore, after splanchnic nerve denervation intramuscular injection of capsaicin into the lateral thigh increased mean pressure by 29 ± 8 mmHg, indicating that animals remained responsive.

**EA at Neiguan-Jianshi, Zusanli-Shangquxu and Guangming-Xuanzhong acupoints (Protocol 2)**

The pressor response in the eight time control rats in the absence of EA was constant over a 90 min period, averaging 31 ± 2 mmHg (Fig. 2A). The inhibitory effects of low frequency EA at the Neiguan-Jianshi (Pe 5-6) and Zusanli-Shangquxu (St 36-37) acupoints were evaluated separately in 13 animals. Baseline blood pressure was reduced slightly but not significantly during the several hours of the experiment. During the control studies, resting MAP varied between 127 ± 16 to 112 ± 13 mmHg (P > 0.05; Fig. 2A), while during the EA protocol, resting MAP ranged between 113 ± 14 and 102 ± 15 mmHg (P>0.05). These variations were not statistically significant. Also, HR in the control group (without EA) was unchanged by gastric distension (370 ± 24 beats/min vs. 372 ± 22
beats/min, before vs. after distension). Before EA at Neiguan-Jianshi or Zusanli-Shangquxu acupoints, the pressor responses were similar to the time control groups, averaging 33 ± 6 and 31 ± 4 mmHg, respectively (Fig. 2B and C). Following 30 min of EA at the Neiguan-Jianshi acupoints in seven rats, the pressor reflex during distension of the stomach was significantly reduced to 18 ± 4 mmHg (-46%), a response that persisted for 40 min after termination of EA (Fig. 2B). EA at the Zusanli-Shangquxu acupoints in six rats yielded similar inhibitory effects, dropping the blood pressure response to 12 ± 5 mmHg after 10 min of EA. This inhibitory response, however, lasted for only 10 min after termination of EA (Fig. 2C). Conversely, EA at the Guangming-Xuanzhong acupoints, did not alter the reflex pressor response induced by gastric distension, which varied between 22 ± 3 mmHg to 26 ± 5 mmHg (Fig. 2D).

**EA during graded gastric distension (Protocol 3)** We noted an increase in mean transmural gastric wall pressure that averaged 5 to 41 mmHg before EA in the six animals distended with volumes of 5 to 25 ml. This range of volumes and associated distension pressures increased mean arterial pressure from 11 to 30 mmHg. Thirty min of EA at the Neiguan-Jianshi acupoints significantly reduced the pressor responses at each level of distension (Fig. 3A) by 35-51% depending upon the infusion volume (-35% with 5 ml and -51% with 15 ml), conversely the volume-pressure relationship was unaltered by balloon distension (Fig. 3B).

**Intravenous naloxone on EA effect (Protocol 4)** EA at the Neiguan-Jianshi acupoints inhibited the reflex response to gastric distension from 33 ± 2 mmHg before to 15 ± 1 mmHg during EA.

However, intravenous naloxone promptly restored the pressor response to 33 ± 4 mmHg, a value not significantly different from the pre-EA control period (Fig. 4), and one which was significantly higher
than the response to EA at the Neiguan-Jianshi acupoints without naloxone (Fig. 2B). Although resting MBP varied between 120 ± 16 and 137 ± 21 mmHg in the protocol, this variation was not statistically significant.

**rVLM naloxone on EA effect (Protocol 5)** Unilateral microinjection of saline (n=6) or naloxone (n=6) into the rVLM was examined in twelve animals. The reflex pressor response in the six saline controls before EA was 23 ± 3.1 mmHg. This response was reduced by 50% to 11 ± 2.9 mmHg after 10 min of EA at the Neiguan-Jianshi acupoints, a response that lasted for 50 min after EA. Before EA, MAP increased by 20 ± 2.6 mmHg during gastric distension in the naloxone treated group. EA at Neiguan-Jianshi acupoints reduced the reflex response to 8 ± 2.6 mmHg (40% decrease. Fig. 5B). Twenty-five min after the onset of EA, unilateral microinjection of naloxone into the rVLM in six rats reversed the blood pressure response to 20 ± 3.4 mmHg, a value similar to that observed before EA. Examination of the rat brain slices verified that all injections located within the rVLM had significantly influenced the reflex responses to gastric distension, while the five injections outside the rVLM (Fig. 6) did not alter the reflex cardiovascular responses. Thus, EA reduced the cardiovascular reflex from 24 ± 5 to 11 ± 4 mmHg while naloxone administered out side the rVLM did not alter this response (8 ± 4 mmHg. However, 50 min after EA the response returned (20 ± 6 mmHg).

**DISCUSSION**

Although several studies (40, 43, 51) have observed variable cardiovascular responses to gastric distention in rats, we were able to develop a model that yielded consistent reflex pressor responses repeatable over a 90 min period. Superimposed on this reflex we showed that low
frequency EA at either the Neiguan-Jianshi or the Zusanli-Shangquxu acupoints significantly reduces the pressor response induced by distension of the stomach in rats. Deutsch (13) reported that rats drink 16 ml of milk within a period of 30 min. This volume was increased to 31 ml when a pyloric cuff was inflated to prevent the stomach from emptying. In the present experiments, gastric transmural wall pressures used to induce pressor reflex were within the range that normally occur in the rat's stomach during feeding (2, 3, 12). Although it might be argued that the rate of infusion of air into the gastric balloon occurred more rapidly than fluid ingestion by rats, we demonstrated inhibition by EA began at very low volumes and pressures (which likely be achieved in a brief periods during drinking). These data therefore reinforce our conclusion that EA has the capacity to inhibit cardiovascular reflex responses over a broad range of gastric wall tensions, including those that likely are achieved during normal eating and drinking activities. These data therefore support our hypothesis that EA inhibits the pressor response to passive distension of the stomach. This modulation of reflex cardiovascular responses by EA is mediated by the endorphin system, since intravenous administration and rVLM microinjection of naloxone, a non-specific opioid antagonist (48), reversed the response.

Distension in the rat’s stomach increases blood pressure, thus providing a new model involving stimulation of mechanoreceptors to study the effects of EA on the cardiovascular responses to reflex activation. Studies in cats have shown that this type of stimulus input can increase blood pressure by approximately 20-25 mmHg (33, 34). Studies in conscious rats have documented increases in blood pressures of >15 mmHg without a change in heart rate following mechanical distension of the stomach (40). Our data are consistent with these earlier findings. In fact, over a 90 min period, gastric stimulation every 10 min resulted in repeatable reflex pressor responses
averaging $31 \pm 2 \text{ mmHg}$. The reflex nature of this response was confirmed by afferent denervation, which virtually eliminated the response.

A potential limitation in this study is the higher baseline blood pressure and slightly lower pressor response in older animals relative to the other groups. For this respect we noted that older rats (e.g. control EA group, Fig. 2D), which generally weigh $> 600\text{g}$ usually had higher baseline blood pressure. We also observed that the distention-induced rise in blood pressure before EA was lower in these older rats. However, the responses of this older group of rats was equivalent to the rVLM-microinjection and splanchnic-denervated animals. Responses in that latter two groups may have been reduced by the additional surgery required in these protocols, including partial craniotomy and bilateral subcostal incisions. However, in all groups we found reproducible responses to gastric distention following surgery. Also, the similarity of base line and pressor responses in all three groups, including one in which EA at Neiguan caused a reversible inhibitory effect (rVLM microinjection group) suggest that a higher baseline blood pressure and lower reflex response did not preclude a response to EA. Therefore, we feel that our data are valid despite the variability of the pressor responses.

The insignificant alterations in heart rate during gastric distension are consistent with the anesthetized state of animals as well as with the presence of an intact baroreceptor system. Anesthetized animals commonly manifest significant parasympatholysis, resulting in high baseline heart rates (38). It also is likely that the baroreceptors reflexly buffer heart rate in response to increases in blood pressure. In fact, the absence of a decrease in heart rate suggests that there was a balance observed between the excitatory reflex responses and the negative chronotropic influence from baroreceptors. Therefore, both anesthesia and an intact baroreceptor system likely contributed to the absence of heart rate changes during mechanical stimulation of the stomach.
Acupuncture in Chinese traditional medicine has been used clinically to treat a variety of diseases including angina pectoris, hypertension and myocardial infarction (5, 6, 9, 14, 41). In previous studies (10, 26, 29) we demonstrated that EA does not significantly affect resting BP. Rather EA is capable of inhibiting reflexly induced pressor responses or hypertension induced by stress or other abnormal conditions. For example, in spontaneously hypertensive rats, and nor-epinephrine infusion-induced hypertensive dogs, EA at acupoints located over the sciatic, deep peroneal and median nerves significantly reduces blood pressure (10, 29, 52). We have found in studies of cats with partial coronary artery occlusion that EA at the Neiguan-Jianshi acupoints overlying the median nerve reduces the ischemic response caused by an imbalance between myocardial oxygen supply and demand during cardiovascular reflex responses provoked by noxious chemical stimulation of the gallbladder (8, 27). The inhibitory effect of EA on the pressor response induced by gastric distension is due to activation of group III myelinated and and group IV unmyelinated somatic afferent fibers during EA (10, 26, 27). The present investigation involves yet another model of sympathoexcitation in rats that may be relevant to conditions such as post-prandial angina in patients with coronary disease, since such stimulation not only augments myocardial oxygen demand but also less frequently results in coronary arterial vasoconstriction (33, 34).

Our purpose of the present study was to compare responses to stimulation of Neiguan-Jianshi, Zusanli-Shangquxu and Gaungming-Xuanzhong acupoints. Likes others (37), we believe that the best control for an acupoint is another acupoint on another meridian that has been reported to have a different function.
In the present study, the Neiguan-Jianshi acupoints were selected because of their depressor effects on elevated blood pressure. The Neiguan-Jianshi acupoints represent sites used in traditional Chinese clinical medicine to treat coronary heart disease and hypertension (4, 5, 6, 9). Also, this acupoint has been useful in studying the effects of acupuncture on the cardiovascular system in animal models (26, 27, 28). The Zusanli-Shangquxu acupoints were chosen since they have been used in Chinese medicine to treat gastrointestinal and immunological diseases (10, 29, 44). The Guangming-Xuanzhong acupoints, belonging to the gallbladder meridian, were chosen as control acupoints. They are considered to be useful in treating leg pain and vision but not cardiovascular dysfunction (44). Therefore, these acupoints provide useful sites to study the issues of mechanism and point specificity of EA. Our results demonstrated that EA stimulation at both Neiguan-Jianshi and Zusanli-Shangquxu acupoints reduced the reflex increase in blood pressure during distension of the stomach. However, the duration of the EA-related effects was not equivalent. EA at the Neiguan-Jianshi acupoint caused substantially more prolonged inhibition of this reflex than EA at the Zusanli acupoint (40-50 vs. 10 min after termination of EA, Fig. 2). In Contrast, EA at Guangming-Xuanzhong did not inhibit the pressor response. This difference in response suggests that there may be point specificity concerning the effect and duration of EA in modifying cardiovascular responses. Traditional Chinese physicians and acupuncturists have long contended that the effectiveness of acupuncture and its derivatives, EA, acupressure, and even moxibustion, are determined to a large extent by the relationship between the acupoint and the underlying clinical disorder receiving therapy with these techniques (9, 28, 31).

We have shown in previous investigations (8, 10, 23, 24) and current study, that the inhibitory effect of EA on the pressor response induced by gastric distention involves interactions between afferent inputs in several regions of the brain. The rVLM is an important integration area for
integration of cardiovascular reflexes. It also appears to serve as a key area for the inhibitory effect of EA on hypertension and defense-reaction-induced pressor responses (26). Afferent inputs from gastric distention pass through the splanchnic nerve to the spinal cord and brain (33, 34). Our recent electrophysiological studies have demonstrated that the afferent inputs from both the splanchnic and median nerves (below the Neiguan and Jianshi acupoints) converge on premotor sympathoexcitatory neurons in the rVLM (49). Thus, excitatory cardiovascular responses induced, for example, by stimulation of splanchnic inputs to the rVLM can be inhibited by EA through stimulation of somatic afferents. This inhibition is due to the activation of µ- and δ- opioid receptors (28) suggesting that endorphins, endomorphin and probably enkephalins are involved in the EA-related modulation of visceral inputs in the rVLM. Recently, Guyenet et al. (20) reviewed the literature documenting that pre-enkephalin mRNA is present in the presympathetic, presumably sympathoexcitatory, cells of the rVLM as well as in GABAergic inhibitory neurons. He also cited evidence suggesting that presympathetic rVLM neurons are controlled by opioid peptides at both pre- and post-synaptic levels. However, the details respect to the action of the opioid system during EA on presympathetic rVLM neurons requires further investigation.

Previous studies have suggested that the influence of EA in the CNS to modulate pain, cardiovascular and other clinical disorders is dependent, at least in part, on the endogenous opioid system (8, 26, 31). Investigations by one of our team members (PI, 10, 26, 29) have demonstrated that both the depressor effect of EA on norepinephrine-induced hypertension and the pressor response induced by carotid chemoreceptor stimulation were blocked completely by naloxone administered intravenously prior to EA. Other studies, relating to the analgesic effect of EA, have shown that naloxone injected before acupuncture prevents the EA-related inhibitory response (21). On the other
hand, we recently have shown that naloxone administered after EA is capable of blocking EA-induced inhibition on the pressor response induced by application of bradykinin to the gallbladder (8, 27). In general, we believe that reversal of a response after it has been induced is a stronger test of opioid hypothesis compared to prevention of a response before it has begun. Therefore, in the present study naloxone was administered intravenously after first demonstrating that EA inhibited the pressor response induced by gastric distention.

Our laboratory also has shown previously that blockade of the opioid system with naloxone administered either intravenously or specifically into nuclei like the rVLM involved with sympathetic outflow suppresses the influence of EA on cardiovascular reflex responses (8, 27). The present study in rats is in accord with these previous studies in cats. Microinjection of naloxone into areas surrounding the rVLM did not block the inhibitory effect of EA on the pressor response induced by gastric distention. In addition to confirming the specificity of opioid receptors in the rVLM, these data show that blockade is not caused by leakage of naloxone into circulation. Because naloxone incompletely inhibited the EA effect, our results suggest that either the dose of naloxone used in this study caused partial blockade or that other neurotransmitters such as serotonin, nitric oxide, and/or γ-aminobutyric acid may be playing a role in the EA-related sympathoexcitatory modulatory effect (18, 29, 30, 53, 55).

We noted a difference in the duration of reversal of the EA response comparing intravenous to rVLM microinjection of naloxone. The shorter influence observed with microinjection may be related to several factors. The half life of naloxone is about 1.1 ± 0.6 hr (16). Therefore, temporary blockade by naloxone likely occurred because naloxone was metabolized or because it was administered unilaterally. We did note that unilateral injection reduced the EA response to a degree comparable to that observed with intravenous administration of naloxone. We suggest that the inhibitory effect of EA requires bilateral input from both the right and left rVLM. When the influence
of either side is removed (i.e., following microinjection of naloxone into one side of the rVLM), the EA effect is largely eliminated. Conversely, naloxone crosses the blood brain barrier and, as such, when administered intravenously, it likely would cause a broader inhibition of the opioid system in regions other than the rVLM. These would include the periaqueductal gray matter, the nucleus arcuatus, and the nucleus raphe obscurus (10, 24, 29). These regions deserve further investigation concerning the influence of EA on the autonomic cardiovascular response. Although a direct comparison between the rVLM microinjection and intravenous injection of naloxone is difficult, both significantly reversed the EA-related modulation of the gastric pressor reflex.

There is clinical importance in reducing the pressor response to mechanical distension of the stomach. Like gastric distension in animals, food ingestion in humans increases heart rate and blood pressure (11, 25). In patients with limited coronary blood supply (e.g. coronary atherosclerosis), food ingestion can provoke postprandial angina resulting from myocardial ischemia (17, 42). The most likely explanation for this phenomenon is that activation of the cardiovascular system, with attendant increases in blood pressure and heart rate, leads to an imbalance between coronary oxygen supply and demand, particularly in the setting of coronary atherosclerosis. Balance between myocardial oxygen demand and supply is vital for normal cardiac contractile function and potentially can be restored by EA (27). According to the results of the present study, stimulation of the Neiguan acupoint appears to be more efficacious than the Zusanli acupoint, although both may prove useful.

In conclusion, low frequency EA at the Neiguan-Jianshi and Zusanli-Shangquxu acupoints modulates the cardiovascular pressor response induced by gastric distension in rats. Distension pressures were within the normal physiological range, suggesting that we evaluated the influence of EA on the reflex cardiovascular response caused by a non-painful stimulus. The influence of EA at the
Neiguan-Jianshi acupoints lasted substantially longer than EA at the Zusanli-Shangquxu acupoint, suggesting some degree of point specificity. In addition, the inhibitory effects of EA were reversed by naloxone, applied either intravenously or by microinjection into the rVLM, indicating that EA works through an opioid-related modulatory influence in the CNS. These data provide a mechanism underlying the action of EA, a technique that may prove to be useful medical treatment in clinical cardiovascular disease.

ACKNOWLEDGMENTS

This project is supported in part by NIH Grant HL 36527, 63313, and Susan-Samueli Center for Complementary and Alternative Medicine.

REFERENCES


21. Han JS. *The neurochemical basis of pain relief by acupuncture*. Beijing: China Medical and Pharmaceutical Technology, 1987,


44. **Shi XM and Zhang MC.** *A Chinese-English dictionary of acupuncture and moxibusion.* Huaxia Publishing House, 1998,


47. **Stux G and Pomeranz B.** *Basics of acupuncture.* Berlin: Springer-Verlag, 1998,


51. **Traub RJ, Sengupta JN and Gebhart GF.** Differential c-fos expression in the nucleus of the solitary tract and spinal cord following noxious gastric distention in the rat.  
*Neuroscience* 74: 873-884, 1996.

52. **Yao T, Andersson S, and Thoren P.** Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized spontaneously hypertensive rats.  

53. **Zhang Z, Lin RJ, Fan W, Qong QL, and Li P.** Involvement of 5-HT and GABA in the inhibition induced by NRO stimulation of RVLM defense-related neurons.  

54. **Zhou X, Vance FL, Sims AL, Screenan CM and Ideker RE.** Prevention of high incidence of neurally mediated ventricular arrhythmias by afferent nerve stimulation in dogs.  

55. **Zhu DN and Li P.** Influence of microinjection of L-arginine and L-NG-monomethyl-L-arginine into the rostral ventrolateral medulla on blood pressure and heart rate in rats.  
Figure legends:

Fig. 1. Mean arterial pressure (MAP) responses to distension of the stomach with 15 ml air (intragastric pressure 20 mmHg) before and after bilateral splanchnic nerve denervation in four rats. Numbers below each bar represent baseline blood pressures before distension. Splanchnic denervation abolished the rise in blood pressure induced by gastric distension verifying that this response represents a reflex. Capsaicin (40 µg) subsequently was injected intramuscularly into the lateral side of thigh to verify that the animals were still responsive.

Fig. 2. Mean arterial pressure (MAP) responses to repeated (every 10 min) distension of the stomach with 15 ml air (intragastric pressure 20 mmHg) in rats. Panel A, time control, in eight rats; Panel B, response to 30 min of electroacupuncture (EA, 1-2 mA, 2 Hz) at the Neiguan-Jianshi
acupoints (pericardial meridian, acupoints 5 and 6, Pe 5-6) in seven rats; Panel C, response to 30 min EA at Zusanli-Shengjuxu acupoints (stomach meridian, acupoints 36 and 37, St 36-37) in six rats; and Panel D, response to 30 min EA at Guangming-Xuanzhong (gallbladder meridian, acupoints 37 and 39, GB 37-39) Times 0-20 min are controls before EA, times 30-50 min are during EA, and times 60-100 are after EA.

Fig. 3. Volume-responses during graded distension of the stomach generated in random order by inflation of balloon with air in six rats. Reflex responses were evaluated before and after EA (1-2 mA, 2 Hz) at Neiguan-Jianshi acupoints. At each volume, EA reduced the mean arterial pressure (MAP) responses (Panel A), but EA did not alter the relationship between balloon volume and transmural gastric pressure (Panel B). -○-: before EA; -■-: after EA at Pe 5-6 for 30 min.

Fig. 4. Mean arterial pressure (MAP) responses to repeated (every 10 min) distension of the stomach with 15 ml air in six rats. Naloxone (4 mg/kg i.v.) reversed the inhibitory effect of EA (1-2 mA, 2 Hz) at Neiguan-Jianshi acupoints.

Fig. 5. Responses of mean arterial pressure (MAP) to repeated distension of the stomach with 15 ml of air in twelve rats. Panel A, raw data of blood pressure recording in one rat. Small arrows indicate distension of stomach. a,b,c,d,e correspond to the time in panel B. a-control; b-ten min after EA; c, after microinjection of naloxone into rVLM; d, 30 min after EA stopped; e-60 min after EA stopped. Panel B, naloxone (4 mg/kg, 100 nl) microinjection into the rVLM of six animals. Panel C, saline control microinjection (0.9%, 100 nl) into the rostral ventrolateral medulla (rVLM) of six animals.
EA (1-2 mA, 2 Hz) was performed at Neiguan-Jianshi for 30 min in both groups. Unilateral microinjection of naloxone in the rVLM transiently reversed the rise in arterial pressure induced by gastric distension.

**Fig 6.** Diagram of sections of the rat medulla illustrating the sites of saline and naloxone microinjection. Injections were unilateral (side chosen randomly). For ease of reading, all injections sites are superimposed on left side of map. Sections are 1.34, 2.00, and 2.50 mm rostral to the obex (35). LGPi, lateral paragigantocellular nucleus; py, pyramidal tract; Amb, ambiguous nucleus; 7, facial nucleus; Sp5 spinal trigeminal nucleus; IO, inferior olive nucleus; MVe, medial vestibular nucleus; SuVe, superior vestibular nucleus; LVe, lateral vestibular nucleus; Sol, Solitary tract nucleus; 4V, fourth ventricle.
FIGURE 1.
FIGURE 4

Naloxone (i.v.)

Δ MAP (mmHg)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>EA Pe 5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>127±17</td>
</tr>
<tr>
<td>10</td>
<td>129±16</td>
</tr>
<tr>
<td>20</td>
<td>132±20</td>
</tr>
<tr>
<td>30</td>
<td>134±18</td>
</tr>
<tr>
<td>40</td>
<td>137±21</td>
</tr>
<tr>
<td>50</td>
<td>129±20</td>
</tr>
<tr>
<td>60</td>
<td>136±18</td>
</tr>
<tr>
<td>70</td>
<td>131±28</td>
</tr>
<tr>
<td>80</td>
<td>135±15</td>
</tr>
<tr>
<td>90</td>
<td>134±24</td>
</tr>
<tr>
<td>100</td>
<td>120±16</td>
</tr>
</tbody>
</table>
FIGURE 5
FIGURE 6

- ○ Saline Injection
- ■ Naloxone Injection
- * Naloxone Injection (outside of rVLM)