Modulation of cardiovascular excitatory responses in rats by transcutaneous magnetic stimulation: role of the spinal cord

Wei Zhou (Y. Syuu),1 Ian Hsiao,2 Vernon W. H. Lin,2 and John C. Longhurst1

1Department of Medicine, Susan SamueLI Center for Integrative Medicine and 2Spinal Cord Injury/Disorder Health Care Group, Department of Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, California

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CARDIOVASCULAR DISEASE REPRESENTS one of the more prevalent causes of morbidity and mortality in middle-aged and older age individuals in both Western and Eastern societies. In particular, substantial morbidity and mortality result from hypertension as a risk factor for ischemic cardiovascular disease. We currently have available in our clinical armamentarium a number of approaches that can be used to treat patients with cardiovascular disorders, including risk factor reduction, pharmacological therapy, and invasive and interventional therapies as practiced by cardiologists and surgeons. Recently, there have been both profound interest and acceptance of a number of complementary therapies. These therapies have emerged because none of the more usual therapies is completely effective in eliminating either the symptoms or adverse outcomes resulting from these diseases. Furthermore, many mainstay therapies are associated with side effects that surprising numbers of patients find unacceptable. Alternative approaches in the medical management of cardiovascular disease have included spinal cord and transcutaneous electrical neural stimulation as well as acupuncture (33). Clinical and experimental studies suggest that acupuncture may be beneficial in severe cardiovascular disease manifestation, including hypertension, arrhythmias, and angina pectoris (3, 4, 11, 22, 48). Each of these conditions can be exacerbated by cardiovascular reflexes that lead to changes in heart rate (HR), blood pressure (BP), myocardial contractility, and/or myocardial oxygen demand during stress.

Our group has demonstrated that low-frequency electroacupuncture (EA) significantly inhibits the cardiovascular sympathetic excitatory reflex responses during gastric distension (12, 23, 48) as well as during chemical stimulation of the gallbladder (9, 22), suggesting that EA potentially can reduce postprandial angina and myocardial ischemia (22). There is interest, however, in identifying other similar therapeutic strategies that are less invasive than acupuncture, because some people do not want needle stimulation. One of these is transcutaneous magnetic stimulation, which is noninvasive and is not painful during induction of relatively small electrical currents in the skin, which has a high resistance. This is in contrast to the passage of large currents delivered percutaneously necessary to excite deep structures with conventional electrical stimulation (13). Magnetic stimulation produces its effect by generating a time-varying magnetic pulse, according to Faraday’s Law, and depolarizes underlying neural pathways (8). Unlike electrical stimulation, which requires direct contact with the tissue, magnetic stimulation can activate deep nervous tissue through a coil placed on the skin’s surface. This technique has been used to stimulate spinal nerves below a region of spinal cord injury to restore vital functions, such as the ability to cough (27, 31), empty the bladder (32), improve colonic transit (29), and produce lower limb muscle contractions (30).

As an alternative to EA, magnetic stimulation potentially can offer a noninvasive method of stimulating somatic nerves to achieve the same beneficial effects as EA (25). For example, magnetic stimulation could evoke antinociceptive effects through the opioid system (25). The underlying mechanism is assumed to be similar to EA (25). Kaufman’s group has shown that intrathecal injection of met-enkephalin attenuates the reflex pressor responses to muscle contraction through inhibition

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Address for reprint requests and other correspondence: W. Zhou (Y. Syuu), Medical Science 1 C240, College of Medicine, Univ. of California, Irvine, CA 92697-4075 (e-mail: wzhou2@uci.edu).

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of substance P release in the spinal cord (20). Met-enkephalin is released in the spinal cord when Aδ- and C-fiber sciatric afferents are stimulated electrically (46). Because EA activates both Aδ- and C-fiber afferents in the median nerve (22, 48), it is reasonable to expect that opioids in the spinal cord may play a role during EA and perhaps during magnetic stimulation. Our previous studies have shown that EA can modulate the cardiovascular reflex responses (12, 23, 48). It would be meaningful to know whether magnetic stimulation has the same modulatory effects on the cardiovascular system as EA and to define mechanisms that underlie the influence of this alternative modality of stimulation.

As shown in our previous studies (12, 23, 48), distension of the rat’s stomach increases BP and provides an appropriate model to study the effects of magnetic stimulation on the cardiovascular reflex activation. Therefore, in the present investigation we investigated the influence of magnetic stimulation on the sympathoexcitatory reflex responses to gastric distension and compared its influence with EA. We tested the following hypotheses: 1) like EA, magnetic stimulation inhibits sympathoexcitatory cardiovascular responses to gastric distension through activation of somatic afferent neural pathways by a mechanism that involves opioid peptides; and 2) μ-, δ-, and κ-opioid receptors in the spinal cord mediate magnetic stimulation-related modulation of cardiovascular reflex responses.

METHODS

Surgical Procedures

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’s Guiding Principles for Research Involving Animals and Human Beings. Studies were performed on adult Sprague-Dawley male rats (400 – 600 g). After an overnight fast (18 h), anesthesia was induced with ketamine (100 mg/kg im) and was maintained with α-chloralose (50 – 60 mg/kg iv). Additional doses of α-chloralose (25–30 mg/kg iv) were given as necessary to maintain an adequate depth of anesthesia. The right jugular vein was cannulated for administration of sodium bicarbonate and α-chloralose. The trachea was intubated, and respiration was monitored with a ventilator (model 661, Harvard Apparatus). The right or left carotid artery was cannulated with a pressure transducer (Statham P23 ID, Gould) to monitor systemic BP. HR was derived from the pulsatile BP signal. In some rats, the median nerve was transected at the upper part of the forelimb. Arterial blood gases and pH were measured periodically with a blood-gas analyzer (ABLS, Radiometer America) 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 and lamp.

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, 14, 15). Within 5–10 s of inflation, we noted an increase in systemic arterial BP. In the single instance when the balloon was not in the stomach but remained in the esophagus, the pressor response was much larger. We eliminated this animal from our data. To evaluate action of opioid subtypes at the spinal cord level, in some animals we inserted a tubing (PE-10) into the spinal subarachnoid space at the level of T4–T5 through an incision made on the atlanto-occipital membrane in the anesthetized rats to block the action of opioids in the dorsal horn at this level (20, 37). At the end of each experiment, we confirmed with Chicago blue dye (10 µl) that the intrathecal injections were confined to T4 and T5.

Experimental Procedures

After the surgical procedure, 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 (48). 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, 48). After the maximal cardiovascular pressor response was observed, air was withdrawn from the balloon. After the completion of each experiment, rats were euthanized with intravenous KCl under deep anesthesia. The stomach was exposed to verify the location of the balloon. BP and HR responses were recorded and analyzed offline with a Pentium III computer and data-acquisition software Power Lab (AD Instruments).

Functional Magnetic Stimulation

Magnetic stimulation was administered by using a Magpro magnetic stimulator (Medtronic) with a butterfly magnetic coil consisting of two circular loops, each 5 cm in diameter. The MagPro magnetic stimulator is capable of generating a maximum field strength of 2.2 Tesla at the center of a round coil. The butterfly magnetic coil allows more focal stimulation, with a maximal induced electric field generated at the center and dropping to zero at about a 3-cm radius (28). The coil was supported by a wood frame, which was adjusted in height so that the center of the coil touched the forearm. Placement was used to ensure that the center of the coil covered the pericardial meridian (P 5–6) acupoints (~1–2 mm proximal to the flex crease of the wrist) overlying the median nerve. A previous study has shown that butterfly-shaped coil can selectively stimulate peripheral nerves (35). The magnetic stimulator was driven by a stimulator through an isolation unit (Grass, model 88) at a frequency of 2 Hz. The stimulation intensity was fixed at 30% of the maximal intensity for all experiments, because we found that this parameter effectively inhibited the reflex response to gastric distension in a preliminary study. Stimulation at 30% of the maximal intensity could be maintained for 8 min before the temperature of the coil exceeded 40°C and stimulation had to be terminated for cooling. We then cooled the coil in ice water for 3 min before resuming stimulation. Magnetic stimulation was repeated three times so that the total duration of stimulation was 24 min for all experiments.

Experimental Protocols

Protocol 1: Magnetic stimulation and EA. TIME CONTROL. Six rats were subjected to nine repeated periods of gastric distension without stimulation. Each distension lasted 30 s and was repeated at 10-min intervals over a 90-min period while reflex BP and HR responses were monitored. Our previous studies concluded that repeatable reflex responses occur with this stimulus paradigm (23, 48).

MAGNETIC STIMULATION. In eight rats, after recording two reproducible control responses to gastric distension, magnetic stimulation (2 Hz, 30% of maximal intensity) was applied unilaterally at the Jianshi-Neiguan (P 5–6) acupoints for 24 min. Each distension was repeated at 10-min intervals over 90 min. Like others (34), we believe that the best control for an acupoint is to stimulate another acupoint on another meridian that has been reported to have a different function. This has been termed the strong control (34). In the present study, we chose Guangming-Xuanzhong (overlying superficial peroneal nerve
above the lateral side of ankle, gallbladder meridian, GB 37–39) as the control acupoints because our previous studies with EA suggest that these acupoints produce little input to cardiovascular centers in the brain stem (44) and does not alter cardiovascular excitatory reflexes (23, 44). Therefore, after recording of two reproducible control responses to gastric distension in five rats, magnetic stimulation was applied at GB 37–39 for 24 min.

EA. After establishment of two consistent BP responses to gastric distension in six rats, 32-gauge stainless acupuncture needles (Suzhou Medical Appliance) were inserted unilaterally into P 5–6 acupoints to a depth of 3–5 mm and were stimulated electrically at 2 Hz, 0.3–0.5 mA, 0.5-ms duration for 24 min to match the duration of magnetic stimulation. Each distension was repeated at 10-min intervals over 90 min. Correct positioning of acupuncture needles in human subjects relies on their feeling of “heaviness” associated with electrical stimulation of the needles when properly placed. However, this information is not available in animals. Therefore, our criterion for correct needle positioning relied on our observation of a slight repetitive flexion of the paw during EA, as in our laboratory’s previous study (48).

Protocol 2: Median nerve denervation. In six control rats, after two reproducible control responses to gastric distension were recorded, the median nerve was transected, and magnetic stimulation was applied (2 Hz, 30% intensity) unilaterally at P 5–6 for 24 min, to determine whether the median nerve serves as the afferent pathway for the magnetic stimulation response.

Protocol 3: Intravenous naloxone. To evaluate the role of the endogenous opioid system in the inhibitory effects of magnetic stimulation, we intravenously administered a nonspecific opioid receptor antagonist, naloxone (Sigma). Naloxone was diluted to a concentration of 4 mg/ml in 0.9% NaCl and a dose of 4 mg/kg (23). Gastric distension was repeated at 10-min intervals over 90 min. Similar to the first protocol, the cardiovascular responses before magnetic stimulation during two separate distensions in six rats were recorded followed by 24 min of magnetic stimulation at P 5–6. Immediately after termination of magnetic stimulation, 4 mg/kg of naloxone were administered intravenously, followed by repeated gastric distension every 10 min over a 40-min recovery period.

Protocol 4: Intrathecal injection of opioid receptor antagonists. We assessed the contribution of the three opioid receptor subtypes (µ, δ, and κ) in the spinal cord, with respect to the inhibitory effect of magnetic stimulation on visceral cardiovascular pressor reflex. Rats were divided randomly into the following groups (n = 6–7 per group) for intrathecal administration of 1) µ-opioid receptor antagonist CTOP (Sigma, 5 nmol in 10 μl it) (47); 2) δ-opioid receptor antagonist, ICI 174,864 (Sigma, 10 nmol in 10 μl) (10); 3) κ-opioid receptor antagonist, nor-binaltorphimine (nor-BNI; Sigma, 10 nmol in 10 μl it) (47); and 4) vehicle control (10 μl saline it). All antagonists were dissolved in saline and administered 10 min before the third magnetic stimulation.

Statistical Analysis

Data are presented as the means ± SE. Mean arterial pressure (MAP) and HR at rest were compared over time using a repeated-measures ANOVA followed post hoc by Tukey’s test. Gastric distension responses also were assessed with one-way repeated-measures of ANOVA followed by Tukey’s test to compare BP responses before, during, and after magnetic stimulation and 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

Magnetic Stimulation and EA

Cardiovascular responses to gastric distension in the time control group were consistent over the 90-min period of evaluation in the absence of magnetic stimulation (Fig. 1A). BP but not HR was increased during mechanical stimulation of the stomach. Twenty-four minutes of magnetic stimulation at the control acupoint GB 37–39 did not alter the reflex increase in BP induced by gastric distension, which varied between 26 and 28 mmHg (Fig. 1B). Conversely, after 24 min of magnetic stimulation at P 5–6, the pressor reflex during distension of the stomach was reduced from 23 ± 5 to 16 ± 4 mmHg, a response that persisted for 10 min after termination of the procedural (Fig. 1C). Baseline MAP was not altered by this maneuver in any of the groups. Twenty-four minutes of low-current, low-frequency (0.3–0.5 mA, 2 Hz) EA at Jianshih-Neiguan (P 5–6) acupoints also inhibited the response (Fig. 1D, P < 0.05). The extent of the inhibitory effects during magnetic stimulation and EA was similar (32 vs. 35%), but the

![Fig. 1. Mean arterial blood pressure (MAP) responses of rats to repeated (every 10 min) distension of the stomach with 8 ml air. A: time control in 6 rats. B: response to 24 min of magnetic stimulation (MS; 2 Hz, 30% of the maximal intensity) at Guangming-Xuanzhong acupoints (gallbladder meridian, GB 37–39) in 5 rats. C: response to 24 min of MS at Jianshi-Neiguan acupoints (pericardial meridian, P 5–6) in 8 rats. D: response to 24 min of electroacupuncture (EA) at P 5–6 in 6 rats. Time 0–10 min are controls before MS or EA, times 20–40 min are during MS or EA, and times 50–80 min are after MS or EA. Numbers below each bar represent baseline blood pressures before distension (means ± SE); n, no. of rats. *Decreased pressor responses compared with the 2 responses before onset of EA or MS, P < 0.05.](http://jap.physiology.org/)
onset of inhibition of EA occurred 10 min earlier and lasted 10 min longer than magnetic stimulation.

Medial Nerve Denervation

The reflex cardiovascular responses to gastric distension in the absence of magnetic stimulation were unchanged by medial nerve denervation (23 ± 6 vs. 24 ± 5 mmHg, before vs. after denervation in four animals). However, denervation abolished the inhibitory effect of magnetic stimulation (Fig. 2B).

Opioid Blockade

In five animals, intrathecal injection of naloxone had no effect on baseline MAP and HR as well as the cardiovascular reflex responses to gastric distension. In another group of six animals in which magnetic stimulation at P 5–6 inhibited the reflex response to gastric distension (22 ± 4 to 13 ± 3 mmHg), we found that intravenous naloxone partially restored the pressor response to 18 ± 4 mmHg. This value was not significantly different from the pre-magnetic stimulation control period (Fig. 2C) and was significantly higher than the response to magnetic stimulation at P 5–6 without naloxone (Fig. 2A). Intrathecal injection of δ- and κ-opioid receptor antagonists, ICI 174,864 and nor-BNI, immediately after termination of magnetic stimulation partly reversed inhibition of the cardiovascular reflex (Fig. 3, C and D). In contrast, the μ-opioid antagonist CTOP failed to alter the cardiovascular reflex responses to gastric distension. 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reflex (Fig. 3B). The small variations in resting MAP of 115 to 121 mmHg in these groups were not statistically significant.

**DISCUSSION**

There are several novel findings in the present study. First, magnetic stimulation at acupoints P 5–6 over the median nerve but not at GB 37–39 over the superficial peroneal nerve inhibits cardiovascular reflex responses to gastric distension by more than 30%, confirming the hypothesis that magnetic stimulation at P 5–6 acupoints, like EA, modulates reflex elevations in BP. Second, magnetic stimulation is about as effective as EA with respect to the extent of its modulatory influence on the reflex sympathoexcitatory responses. Third, median nerve denervation abolishes the modulatory effect of magnetic stimulation, suggesting that this somatic afferent pathway plays an important role in mediating cardiovascular inhibition by magnetic stimulation. Fourth, the general opioid receptor antagonist naloxone blocked the inhibitory effect of magnetic stimulation, demonstrating an involvement of an opioidergic mechanism for magnetic stimulation-related cardiovascular inhibition. Finally, in contrast to our hypothesis, intrathecal injection of δ- and κ-opioid receptor antagonists at T5–T6, but not the μ-opioid antagonist, reversed the inhibition of the cardiovascular reflex by magnetic stimulation, indicating that opioid receptors in the thoracic spinal cord contributes to this modulation.

Our previous studies in cats and rats demonstrate that selective stimulation of the acupoints by EA through fine needle insertion can reduce sympathoexcitatory reflex responses by 30–50% (12, 23, 44, 48). However, there are no previous studies on the effect of transcutaneous magnetic stimulation in this respect. In the present study, we chose P 5–6 and GB 37–39 to determine the effect of magnetic stimulation on point specificity. We have demonstrated previously that P 5–6 presents an active acupoint, whereas GB 37–39 can serve as a control acupoint in EA-related modulation of cardiovascular reflex response (23, 44). Similarly, magnetic stimulation directed generally at P 5–6 acupoints caused prolonged inhibition of this reflex, whereas magnetic stimulation at GB 37–39 did not inhibit the pressor response. This difference in response suggests that, like EA (23, 44), there is point specificity (i.e., afferent nerve selectivity) concerning the effect of magnetic stimulation in modifying cardiovascular responses.

We recently demonstrated that stimulation of the median nerve either by EA or by manual acupuncture can significantly inhibit the reflex excitatory cardiovascular responses by activating Aδ- and C-fiber somatic sensory nerves (43, 48). This study therefore showed that thin-fiber afferents provide important input to the central nervous system during both forms of acupuncture. Magnetic stimulation can effectively stimulate both superficial and deep peripheral nerves, as well as neurons in the motor cortex of the brain (1, 5, 6) and motoneurons in the spinal cord to evoke muscular contractions of expiratory muscles and bowel and bladder musculature (26, 27). Magnetic stimulation also has been shown to activate primary afferents and could evoke an antinociceptive effect (25). In preliminary studies, we attempted to record action potential in the median nerve using single-unit recordings to identify the neural fiber types activated by magnetic stimulation. However, this is not possible because of the interference by the large broad stimulation artifact. We observed that both magnetic stimulation and EA inhibited the pressor reflex responses to gastric distension, but the duration of the inhibitory BP responses by EA is longer than with magnetic stimulation. One possible difference may be related to the degree of somatic afferent input during EA vs. magnetic stimulation. Unfortunately, with the broad stimulation artifact, we were unable to record afferent activity. We recently have demonstrated, using the technique of neonatal capsaicin administration, that group IV somatic afferent pathways contribute to the EA-related cardiovascular response (43). Future studies involving this technique could be used to identify the role of unmyelinated somatic afferents in the magnetic stimulation-BP lowering responses.

We were unsure whether the response to magnetic stimulation would be mediated by one or more neural pathways (e.g., radial and median nerves) because the ability to focus the area of stimulation is not as great as with EA using needle stimulation. However, transection of the median nerve abolished this inhibitory effect, indicating that the median nerve serves as the principal afferent pathway for the magnetic stimulation-related cardiovascular regulation.

Naloxone is a nonspecific opioid receptor antagonist. Although it is relatively more μ-receptor selective, it is active to some extent in blocking all three of these opioid receptors, including the μ, δ, and κ subtypes (42). We found that intrathecal naloxone in the absence of magnetic stimulation had no effect on the reflex cardiovascular response to gastric distension. This finding is similar to those reported by several other investigators. For example, Pomeroy et al. (38) found that intrathecal naloxone had no effect on the cardiovascular responses to exercise in conscious dogs. In addition, Waldrop and Iwamoto (45) found that intravenous naloxone had no effect on the reflex cardiovascular responses to static contraction in decerebrate cats. However, intrathecal naloxone partially reversed the inhibition by magnetic stimulation, suggesting a role for the endogenous opioid system during afferent stimulation by this intervention. The present study further demonstrated that both δ- and κ- but not μ-opioid receptor antagonists blocked the inhibitory effect of magnetic stimulation on the reflex BP response induced by gastric distension. The inhibitory effects of the δ- and κ-receptor antagonists, ICI 174,864 and nor-BNI, were strong and similar in magnitude, each lasting for more than 20 min. Enkephalins stimulate δ-opioid receptors (40), whereas dynorphin have a strong affinity for κ-opioid receptors (40). Thus our study suggests that enkephalins and dynorphin, but not β-endorphin, in the spinal cord likely play a significant role in the magnetic stimulation-mediated regulation of cardiovascular reflexes. These findings are in line with the previous reports that EA works through enkephalins and dynorphin but not β-endorphin in the spinal cord (18, 19). Interestingly, these findings are different from our previous study showing that μ- and δ-receptor but not κ-opioid receptors in the rostral ventral lateral medulla are involved in the modulation of reflex sympathetic activity by EA (24). Different opioid systems at brain stem and spinal cord levels appear to contribute to the modulation by somatic afferent stimulation (i.e., during EA and magnetic stimulation).

Anatomical and physiological studies indicate that the dorsal horn of the spinal cord serves as a major center for opioid-induced analgesia. In this respect, Hokfelt et al. (21) have shown that enkephalin-containing neurons and nerve terminals...
are present in laminae I and II of the dorsal horn. Dynorphin coexists with enkephalin in some dorsal horn neurons (7). δ-Opioid agonists inhibit excitatory transmission in spinal dorsal horn neurons (17). δ-Receptor binding sites are mainly found in cervical and thoracic segments of the cord, whereas κ sites are located principally in the lumbosacral spinal cord. Like enkephalin, δ- ligands are most important in modulation of noiception induced by thermal stimulation at hind paw in rats, whereas κ-agonists exclusively modulate visceral noiception (39).

It is well recognized that mechanical and chemical stimulation of abdominal visceral organs, including the stomach, gallbladder, and pancreas, reflexly excite the cardiovascular system (36). Our previous studies have shown that endogenous substance P and excitatory amino acids such as glutamate acting as sensory neurotransmitters are involved in the visceral excitatory cardiovascular reflex caused by chemical stimulation of gallbladder in the spinal cord (16, 37). Stimulation of Aδ- and C-fiber afferents can cause the release of met-enkephalin in the spinal cord (46). It is likely that the opioid system triggered by magnetic stimulation modulates the release and/or action of other neurotransmitter systems such as glutamate and/or substance P. However, further investigation is required to fully define the role of opioids in the spinal cord during magnetic stimulation or EA.

The ability of transcutaneous magnetic stimulation to reduce the reflex sympathoexcitatory response to mechanical distention of the stomach may be clinically important. Food ingestion in humans increases HR and BP (14). In patients with limited coronary blood supply (e.g., coronary atherosclerosis), food ingestion can provoke postprandial angina resulting from myocardial ischemia (41). The most likely explanation for this phenomenon is that activation of the cardiovascular system, with attendant increases in HR and BP, leads to an imbalance between coronary oxygen supply and demand, particularly in the setting of coronary atherosclerosis. The balance between myocardial oxygen demand and supply is vital for normal cardiac function and potentially can be restored by EA (22). The present study demonstrates that a noninvasive magnetic stimulation might also be a useful treatment of cardiovascular diseases as an alternative to acupuncture.

In conclusion, magnetic stimulation over the P 5–6 acupoint modulates the cardiovascular excitatory response induced by gastric distension in rats. The influence of EA at P 5–6 acupoints lasted longer than magnetic stimulation, suggesting differences in somatic input to the central nervous system. The inhibitory effects of magnetic stimulation were abolished by median nerve transection, indicating that this nerve serves as the somatic afferent pathway in this response. The inhibitory effect of magnetic stimulation was reversed partially by intravenous naloxone as well as by δ- and κ- but not by μ-opioid receptor blockade at T₄–T₅ in the spinal cord, suggesting that opioids, especially enkephalins and dynorphin, contribute to the magnetic stimulation-mediated modulation of cardiovascular reflex responses.

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