

Naloxone reverses inhibitory effect of electroacupuncture on sympathetic cardiovascular reflex responses

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Naloxone reverses inhibitory effect of electroacupuncture on sympathetic cardiovascular reflex responses. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H2127–H2134, 1999.—Acupuncture and electroacupuncture (EA) have been used in traditional Chinese medicine to treat a wide range of diseases and conditions, including angina pectoris and myocardial infarction. In a feline model of reflex-induced reversible myocardial ischemia, electrical stimulation of the median nerves to mimic EA (Neiguan acupoint) significantly improved ischemic dysfunction, secondary to an inhibitory effect of EA on reflex pressor effects evoked by bradykinin (BK). The central mechanism of EA's inhibitory effect in this model is unknown. Accordingly, in α -chloralose-anesthetized cats, BK (10 μ g/ml) was applied to the gallbladder to elicit a cardiovascular reflex response that significantly ($P < 0.05$) increased arterial blood pressure and heart rate; normalized systolic wall thickening (%WTh) of the left ventricle, measured by ultrasonic single-crystal sonomicrometer, increased by $31 \pm 11\%$ ($P < 0.05$). After ligation of a side branch of the left anterior descending coronary artery, the reflex pressor response to BK resulted in a significant decrease of %WTh ($-32 \pm 6\%$) in the ischemic region. When bilateral EA of the Neiguan acupoints was performed, the pressor response to BK was inhibited and regional myocardial function was significantly improved ($+19 \pm 20\%$). The inhibitory effects of EA on blood pressure and %WTh were reversed by intravenous injection of naloxone (0.4 mg/kg; $n = 9$) or microinjection of naloxone (10 nM in 0.1 μ l/site; $n = 14$) into the rostral ventrolateral medulla (rVLM). Thus %WTh with intravenous naloxone was reduced to $-13 \pm 29\%$ ($P < 0.05$) during stimulation of the gallbladder. Our results indicate that the inhibitory effect of EA on the BK-induced pressor response and the consequent improvement of ischemic dysfunction is dependent on the activation of opioid receptors, specifically receptors located in the rVLM.

pressor response; myocardial ischemia; rostral ventrolateral medulla; systolic wall thickening

EPIDEMIOLOGIC STUDIES have demonstrated a relationship between gallbladder disease and coronary artery disease (5, 39). Removal of a diseased gallbladder can reduce angina pectoris or electrocardiographic irregularities (27, 41). The diseased gallbladder likely produces bradykinin (BK), because BK is known to be an inflammatory mediator produced by visceral organs

(42). In this regard, previous experiments (26, 29, 30) indicated that application of BK to the serosal surface of the gallbladder stimulates chemosensitive endings of afferent fibers that travel in splanchnic nerves to reflexly activate the cardiovascular system. The cardiovascular response includes a pressor response, tachycardia, and increases in left ventricular pressure (LVP) and the first derivative of LVP (LV dP/dt), responses that can evoke myocardial ischemia in hearts with a compromised coronary circulation (18).

In traditional Chinese medicine, acupuncture has been used for centuries to treat a variety of diseases and disorders (22, 42). Clinical evidence indicates that acupuncture may have therapeutic effects on some types of hypertension, coronary heart disease, arrhythmias, angina pectoris, and myocardial infarction (2, 8, 10, 33, 38). Studies in anesthetized animals (19) also have demonstrated beneficial effects of acupuncture and electroacupuncture (EA) on myocardial ischemia, arrhythmias, hypertension, and hypotension. Recently, our laboratory (18) found that the cardiovascular responses and the resultant myocardial ischemia caused by the increase in myocardial oxygen demand during application of BK on the gallbladder in cats with partial occlusion of the coronary artery could be inhibited by EA-like bilateral stimulation of the median nerve.

The effects of EA on the cardiovascular system likely are the result of excitation of group III and possibly group IV afferent fibers beneath the acupoint (18, 21). Previous work has demonstrated that such afferent inputs can activate a sympathetic inhibitory system in the brain, resulting in the release of endogenous opioids, γ -amino-*n*-butyric acid and serotonin (17). These mediators can inhibit sympathetic neurons in the nucleus paragigantocellularis lateralis (PGL) of the rostral ventrolateral medulla (rVLM) (14, 15, 17), an important center responsible, in part, for maintaining blood pressure (BP) and integrating cardiovascular reflexes (3, 36).

The presence of opioid receptors in the central nervous system (CNS) (12), including the rVLM (6, 28), and their role in regulation of the cardiovascular system have been reported previously (6, 28, 32, 37). Previous work by one member of our group (37) has shown that microinjection of opioid agonists into the rVLM steadily decreases BP and heart rate (HR) in normotensive, chronic stress-induced hypertensive and spontaneously hypertensive rats, whereas naloxone injected into the rVLM blocks this effect (37). Others

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have reported that injection of opioid agonists into the rVLM inhibits the pressor responses to carotid occlusion (32) and muscular contraction (6). However, there is no evidence at present to implicate the endogenous opioid system in the rVLM in the acupuncture-induced inhibition of myocardial ischemia provoked by reflex sympathetic stimulation of the nervous system.

In the present study, therefore, we hypothesized that the mechanism of the inhibitory effect of EA on the gallbladder-induced pressor response and on regional demand-induced cardiac ischemia is opioid related and occurs in the PGL of the rVLM. To investigate this hypothesis, we utilized percutaneous EA in our feline model of repeatable, reversible, reflex-induced myocardial ischemia, before and after the administration of an opioid antagonist, naloxone, either intravenously or by microinjection into the PGL. A preliminary report on this work has been presented (7).

METHODS

Surgical Preparation

Studies were performed on adult cats of either sex (2.4–4.7 kg). Anesthesia was initially induced with ketamine (40 mg/kg im). The right femoral vein was cannulated to enable administration of α -chloralose (50 mg/kg) and other drugs. Supplementary doses of α -chloralose (10 mg/kg iv) were given whenever necessary to maintain an adequate depth of anesthesia, as assessed by lack of response to noxious toe pinch, a respiratory pattern that followed the ventilator, and stable BP. Intubation of the trachea was performed for artificial ventilation. Arterial blood gases and pH were measured frequently with a blood gas analyzer (model M168, Shanghai Medical Analytic Instrument Factory, Shanghai, PRC) and maintained within the normal range (P_{CO_2} 32–35 mmHg; P_{O_2} >100 mmHg) by enriching the inspired O_2 supply and adjusting the ventilatory rate or volume. Arterial pH was kept between 7.32 and 7.43 and was corrected as necessary by the infusion of 8% sodium bicarbonate. Body temperature was monitored with a rectal probe and was maintained between 36.5 and 37.5°C by a thermostatically controlled heating pad. Systemic BP was measured through a cannula inserted into the femoral artery, which was connected to a pressure transducer (model TP-101T, Nihon Kohden, Tokyo, Japan). Mean arterial pressure (MAP) and HR were derived from the pulsatile signal of the BP waveform. A polyethylene tube was inserted into the left ventricle (LV) through the left common carotid artery to measure LVP; dP/dt was obtained by processing the pressure signal with a derivative amplifier.

A midline laparotomy permitted exposure of the gallbladder. During surgical procedures in the chest or brain, the abdominal wall was closed again with a clip to maintain moisture in the abdominal cavity and to prevent heat loss. The abdomen was opened again only when BK or saline was applied to the gallbladder.

Protocol 1

In 12 cats, a median or near-midline sternotomy was performed. The pericardium was incised carefully and sutured to the chest wall to expose the heart and anterior coronary artery. A small, high diagonal branch of the left anterior descending coronary artery (LAD) on the anterior LV wall was identified, and a 6-0 surgical silk suture was passed below the vessel for subsequent ligation. A crystal transducer of the single-crystal sonomicrometer system (31) was fixed

with tissue adhesive (3M, Neuss, Germany) to the anterior wall of the LV in the region supplied by the diagonal branch of the LAD. Regional LV wall motion was measured pre- and postligation using the sonomicrometer system. Care was taken to prevent drying of the exposed anterior surface of the heart by covering it with a saline-moistened gauze. Technically satisfactory wall motion data were obtained in 9 of the 12 animals.

Protocol 2

In 23 cats ($n = 14$ experimental, $n = 9$ vehicle control), a craniotomy was performed to expose the ventral surface of the medulla for microinjection. In these animals, to minimize the trauma of surgery, a sternotomy was not performed. The trachea and esophagus were retracted, and the prevertebral muscles were removed from the basal plate of the skull. The basioccipital bone was carefully removed from the atlanto-occipital membrane with rongeurs, and the craniotomy was extended for ~4 mm on each side of the midline over the medullary surface. The dura then was cut, and the cerebrospinal fluid was removed, exposing the surface of the medulla. Warm paraffin oil was used to cover the exposed medullary surface to prevent desiccation. The animal's head was fixed in a stereotaxic frame (model JW-1C, Second Military Medical University, Shanghai, PRC) to allow microinjections to be made into the rVLM.

Acupuncture needles (Suzhou Acupuncture Medical Appliance, Suzhou, PRC) were inserted at the Neiguan acupoint overlying the median nerve to a depth of 5–10 mm. An electrical stimulator with a stimulus isolation unit (model JL-B, Shanghai Jialong Teaching Instrument Factory, Shanghai, PRC) provided current to the needles. Correct positioning was confirmed by observing slight repetitive paw flexion during stimulation.

Chemicals

BK (Sigma, St. Louis, MO) was dissolved in normal saline at room temperature to an initial concentration of 2.5 mg/ml. Appropriate serial dilutions were made to obtain desired concentrations. The concentration of the final solution of BK was 10 μ g/ml, because this was the lowest concentration that consistently yields maximal cardiovascular responses when applied to the gallbladder (30). The stock solution of BK was stored in a freezer and was used for no more than 2 wk before a fresh stock solution was prepared. Naloxone hydrochloride (Sigma) also was dissolved in normal saline. The concentration was 0.4 mg/ml for intravenous injection or 10 nM for microinjection into the PGL of the rVLM.

Microinjection Technique

For bilateral microinjection into the rVLM, stainless steel guiding tubes [outside diameter (OD) = 0.46 mm, internal diameter (ID) = 0.27 mm] were positioned at the surface of the medulla at a point overlying the PGL, as indicated by axis coordinates (2.5–3.5 mm lateral to the midline, 0.5–1 mm below the ventral surface of the medulla, and 0.5–1.5 mm caudal to the trapezoid border) (11, 24). An injection cannula (OD = 0.23 mm, ID = 0.11 mm) connected to a 0.5- μ l microsyringe (model W-101, Shanghai Medical Laser Instrument Factory, Shanghai, PRC) with a polyethylene catheter (PE-10) then was lowered through the guiding tubes. The injection cannulas extended 1.0 mm beyond the guiding tubes. The volume of injection (100 nl for each site) was administered in ≤ 1 min. After injections were completed, the cannula was always allowed to remain in the brain for 4–5

min to prevent possible backflow of the solution along the injection track.

Histology

At the end of each microinjection experiment, 0.1 μ l of 0.5% pontamine sky blue was applied to each injection site. After the animal was killed, the brain stem was removed and fixed in 10% Formalin. Frozen serial sections (50 μ m) of the brain were cut with a freezing microtome (model CM1900-Kryostat, Leica, Wetzlar, Germany). The slices were stained with neutral red, and the injection site was identified by microscopic examination.

Protocols

A minimum of 1 h for stabilization of the preparation was allowed before observations were begun. BK (10 μ g/ml) was applied onto the serosal surface of the gallbladder with a 1-cm² pledget of filter paper soaked in the solution of BK. After maximal cardiovascular responses were evoked, the filter paper was removed and the gallbladder was washed at least three times with saline-immersed cotton-tipped applicators to remove excess BK. A significant reflex response was considered to have occurred if BP increased >10 mmHg (26). The average increase in mean BP was 40 ± 5 mmHg after the application of BK. To prevent tachyphylaxis to BK, recovery periods of at least 15 min were provided after each application of BK; this procedure allowed consistent, repeatable responses. As a control for the vehicle, it was demonstrated that cold saline did not evoke any cardiovascular responses when applied to the gallbladder.

Protocol 1. After repeatable baseline responses to BK were recorded, a small branch of the LAD was ligated and two additional applications of BK were performed during a 30-min period. Bilateral EA at the Neiguan acupoint (median nerve) was then applied (2–5 V, 0.5-ms pulse duration, and 4 Hz) for 30 min. During this period, repeated cardiovascular responses to BK were induced at 10 and 25 min. After EA was terminated for 5–10 min, naloxone was administered by intravenous injection (0.4 mg/kg). Subsequently, cardiovascular responses to BK were induced at least twice at 15-min intervals. Using a similar protocol, our laboratory previously has shown (18) in time-control animals that the inhibitory effect of EA-like stimulation at the Neiguan acupoint persists for at least 1 h.

Protocol 2. A protocol similar to that of *protocol 1* was followed except that the chest was not opened and ischemia was not induced. In this preparation, the coronary artery was not ligated and wall motion was not assessed. Instead, BP was used as the measure of the reflex cardiovascular response. After EA had been stopped for 5–10 min, naloxone was administered by bilateral microinjection (10 nM in 0.1 μ l) into the rVLM. In vehicle control animals, the same protocol as *protocol 1* was used except that normal saline rather than naloxone was microinjected.

Data Measurement

Systemic BP, MAP, HR, LVP, LV dP/dt, and LV wall motion were recorded on an eight-channel polygraph (model RM-6000, Nihon Kohden, Tokyo, Japan) and simultaneously input into a computer (Pentium-133, IBM) in some experiments. Systolic BP (SBP), diastolic BP (DBP), and maximal dP/dt were calculated from recorded data. From regional LV wall motion, systolic wall thickening was calculated as $WTh = 100 \times [(ESD - EDD)/EDD]$, where ESD is end-systolic dimension, calculated from the end of the T wave or 20 ms before peak $-dP/dt$, and EDD is end-diastolic dimension,

calculated from the onset of dP/dt. Normalized WTh (%WTh) was calculated as $100 \times [(\text{maximum WTh response to BK} - \text{pre-BK WTh})/\text{pre-BK WTh}]$ (18). Data were collected after each application of BK: baseline (typically 2–3 applications or until responses were consistent), twice during ligation pre-EA (*protocol 1*), twice during ligation + EA (or EA alone, *protocol 2*), and at least twice after EA was stopped.

Statistical Analysis

All data are presented as means \pm SE. The assumption of normal data distribution was assessed with the Kolmogorov-Smirnoff test. The Student's paired *t*-test with a Bonferroni correction was used to statistically compare myocardial wall thickening at rest and at peak BK effect at several time points. Statistical comparisons among multiple groups were made using a repeated-measures analysis of variance (ANOVA). The Student-Newman-Keuls post hoc test was used to test the significance of three preselected comparisons in *protocol 1*: 1) ligation versus baseline, 2) ligation + EA versus ligation, and 3) naloxone versus ligation + EA. In *protocol 2*, the preselected comparisons were 1) EA versus baseline and 2) naloxone (or saline) versus EA. Unless otherwise stated, the comparisons utilized the second BK application during each measurement period. When the normality test failed, a repeated-measures ANOVA on ranks followed by the Student-Newman-Keuls method was used for pairwise multiple comparisons. $P < 0.05$ was considered to be significant.

RESULTS

Effect of Intravenous Naloxone on EA Response (Protocol 1)

Baseline hemodynamic parameters. In *protocol 1* animals, baseline SBP, DBP, MAP, and HR were 159 ± 7 mmHg, 87 ± 6 mmHg, 110 ± 6 mmHg, and 181 ± 9 beats/min, respectively. Coronary ligation, EA, and intravenous naloxone did not significantly affect these resting parameters ($P > 0.05$) except for a small, statistically significant ($P < 0.05$) reduction in HR during ischemia (172 ± 8 beats/min).

Pressor response evoked by BK on gallbladder. The application of BK to the serosal surface of the gallbladder resulted in pronounced activation of the cardiovascular system. This was characterized by marked elevation of BP (Fig. 1), LVP, and dP/dt (data not shown). After coronary artery ligation, the hemodynamic changes evoked by BK were not significantly decreased (except for SBP), suggesting that ligation of a small branch of the LAD does not significantly affect the cardiovascular responses evoked by BK (Fig. 2). EA significantly reduced the reflex pressor response, particularly after 25 min (Fig. 2). The reflex increases in SBP and DBP during ischemia (32 ± 5 and 28 ± 6 mmHg, respectively) were significantly decreased by EA (19 ± 3 and 12 ± 2 mmHg, respectively). Reflex increases in HR were small and were not affected by ischemia or EA.

Ten minutes after the administration of naloxone intravenously, the hemodynamic changes evoked by BK increased; after 25 min, the BK-induced increments of SBP, DBP, and MAP were significantly increased compared with pre-naloxone values (Fig. 2), suggesting

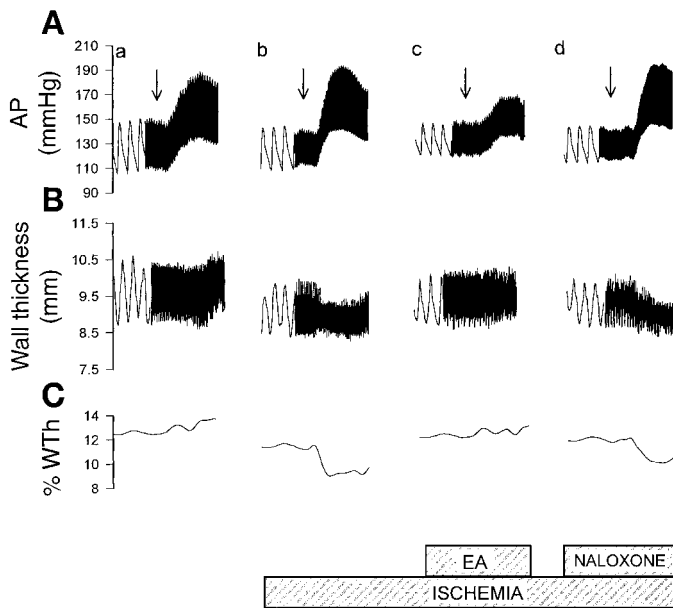


Fig. 1. Computer-captured recordings of reflex increases in arterial blood pressure (AP; A) and myocardial wall thickening (B and C). At points indicated by arrows, a pledget soaked in bradykinin (BK; 10 μ g/ml) was applied to the serosal surface of the gallbladder: a, baseline control measurement; b, a small branch of left anterior descending coronary artery (LAD) was ligated and response to BK was examined after 30 min; c, electroacupuncture (EA; 0.5 ms, 4 Hz) was applied at Neiguan acupoint in foreleg for 30 min and BK was applied at 25 min; d, naloxone was administered intravenously (0.4 mg/kg) after 5–10 min and response to BK was measured 25 min later. Fall in normalized wall thickening (%WTh) associated with reflexly increased sympathetic stimulation in presence of LAD ligation (b) was reversed by EA (c). A decline in AP response also was apparent (c). Intravenous administration of the opioid antagonist naloxone blocked effects of EA; wall thickening declined, and AP response returned to normal (d). We have shown previously that sympathoinhibitory effects of EA at Neiguan persist for at least 1 h (18). Therefore, naloxone was administered at a time when sympathoinhibition by EA would be expected to maintain improved %WTh and diminished AP response to BK.

that the inhibitory effect of EA on the cardiovascular responses evoked by BK could be reversed by an opioid antagonist.

Regional ventricular function. At baseline, the application of BK to the gallbladder significantly ($P < 0.05$) increased WTh from its $11 \pm 1.1\%$ resting value to $14 \pm 1.4\%$ (Fig. 3). After ligation of the diagonal branch of the coronary artery, resting WTh ($11 \pm 1.1\%$) was unchanged, but %WTh during the pressor response to BK decreased markedly to $-32 \pm 6.2\%$ ($P < 0.05$). After EA, %WTh increased significantly to $+19 \pm 20\%$ (resting WTh = $8.0 \pm 1.1\%$). Intravenous naloxone significantly reversed the regional contractile response to BK ($-13 \pm 29\%$).

Effect of rVLM Naloxone on EA Response (Protocol 2)

Baseline hemodynamic parameters. In 14 cats, resting SBP, DBP, MAP, and HR were 157 ± 12 mmHg, 89 ± 8 mmHg, 114 ± 9 mmHg, and 183 ± 8 beats/min, respectively. EA and microinjection of naloxone did not affect these parameters ($P > 0.05$). The baseline hemodynamic parameters in this group generally were simi-

lar to those observed in the group receiving naloxone intravenously.

Pressor response evoked by BK on gallbladder. In comparison with the intravenous naloxone group, this group showed similar activation of the cardiovascular system when BK was applied to the gallbladder. In the 25th minute of EA, all hemodynamic changes evoked by BK were significantly decreased in comparison with control (Fig. 4). Reflex-induced increments of SBP and DBP decreased from control (45 ± 7 and 38 ± 6 mmHg, respectively) to 22 ± 6 and 23 ± 6 mmHg, respectively, during EA. Microinjection of naloxone bilaterally into the PGL of the rVLM did not change resting hemodynamic parameters, whereas the changes in SBP, DBP, and MAP induced by BK at 5 min after microinjection were significantly larger than the increments observed before naloxone. Reflex increases in SBP and DBP were 37 ± 6 and 32 ± 6 mmHg, respectively, after naloxone ($P < 0.05$). In comparison, saline control animals showed no change in MAP at the time point corresponding to naloxone injection (Fig. 4).

Microinjection sites. Examination of the brain slices revealed that all of the injection sites were within the PGL of the rVLM (Fig. 5).

DISCUSSION

This is the first study to show that naloxone, administered intravenously or microinjected into the rVLM, significantly reverses the inhibitory effect of EA on the reflex increase in arterial BP after the application of BK to the gallbladder. Naloxone also exacerbated regional cardiac dysfunction associated with imbalance

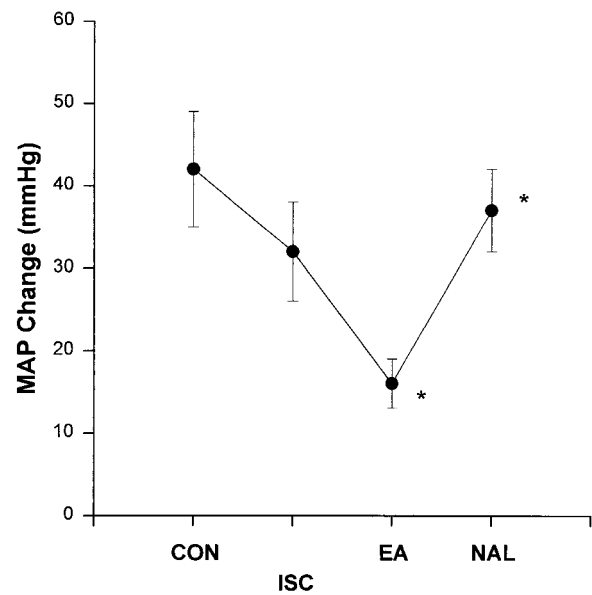


Fig. 2. Effects of coronary ischemia (Isc), EA, and naloxone (Nal) on mean arterial blood pressure (MAP) changes produced by BK. After repeatable control (Con) responses to BK were obtained, a small branch of LAD was tied. BK applied to the gallbladder produced a nonsignificant reduction in blood pressure response. Bilateral EA for 25 min produced a significant reduction of pressor response, which was reversed by naloxone (0.4 mg/kg iv). Data are means \pm SE; $n = 10$. * $P < 0.05$.

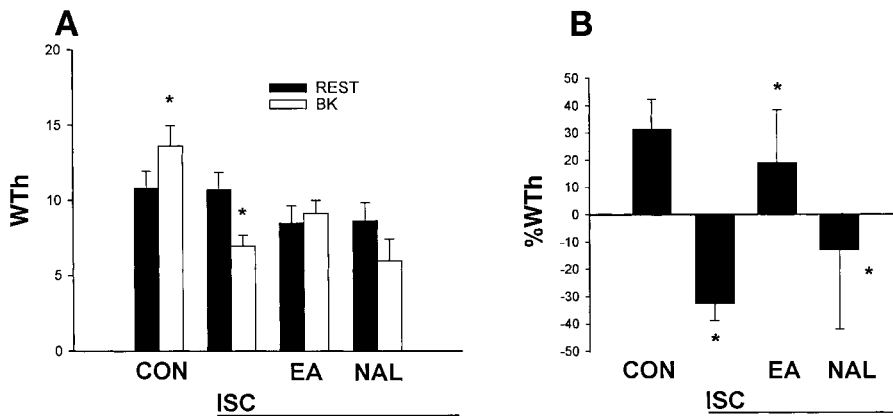


Fig. 3. A: effect of EA on regional systolic WTh of left ventricle. A small branch of LAD was ligated, and demand-induced ischemia was provoked by application of BK to gallbladder to elicit a sympathetic response. Data are means \pm SE; $n = 9$. * $P < 0.05$, rest vs. peak BK response. B: %WTh at 4 time points shown in A. Significant decline in wall motion during ischemia was significantly reversed by EA. Administration of Nal (0.4 mg/kg iv) significantly reduced effect of EA. Data are means \pm SE. * $P < 0.05$, Isc vs. Con, EA vs. Isc, and Nal vs. EA.

between myocardial oxygen supply and demand. These data support our hypothesis that the effects of EA in this model are mediated by opioids, specifically those released in the rVLM.

In traditional Chinese medicine, EA has been used to treat a wide range of diseases and disorders, including hypertension, angina pectoris, myocardial infarction, and cardiac arrhythmias (2, 8, 10, 33, 38). Animal experiments have confirmed that EA has therapeutic effects in hypertension, hypotension, and myocardial ischemia (19). For example, stimulation of somatic afferents in the sciatic nerve to mimic EA depresses BP in nonanesthetized spontaneously hypertensive rats (45) and inhibits the periaqueductal gray-evoked pressor response (25). In the present study, we found inhibitory effects of EA on the reflex pressor response induced by the application of BK to the gallbladder in a

manner similar to the long-lasting sympathoinhibitory response to EA described by Lovick et al. (25). Previous work by one member of our group (15) has shown that EA in normotensive rabbits activates a sympathetic inhibitory pathway in the brain, including the arcuatus

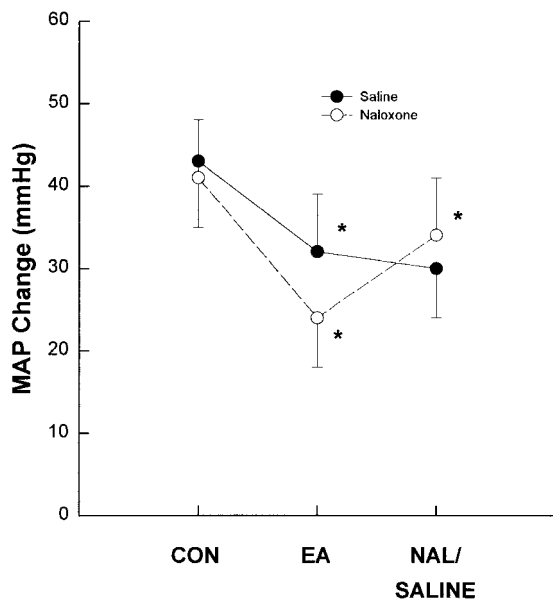


Fig. 4. Comparison of hemodynamic effects of Nal (0 nM in 0.1 μ l; $n = 14$) and normal saline (0.1 μ l; $n = 9$) microinjected into rostral ventrolateral medulla after EA. EA significantly reduced reflex pressor response to BK applied to gallbladder. Nal administration significantly blocked effect of EA, which was not altered by vehicle (saline), indicating a persistent effect of EA on blood pressure that could be blocked, in part, by Nal. Data (means \pm SE) for Nal and saline injections were obtained 5 min after administration. * $P < 0.05$, EA vs. Con and Nal vs. EA.

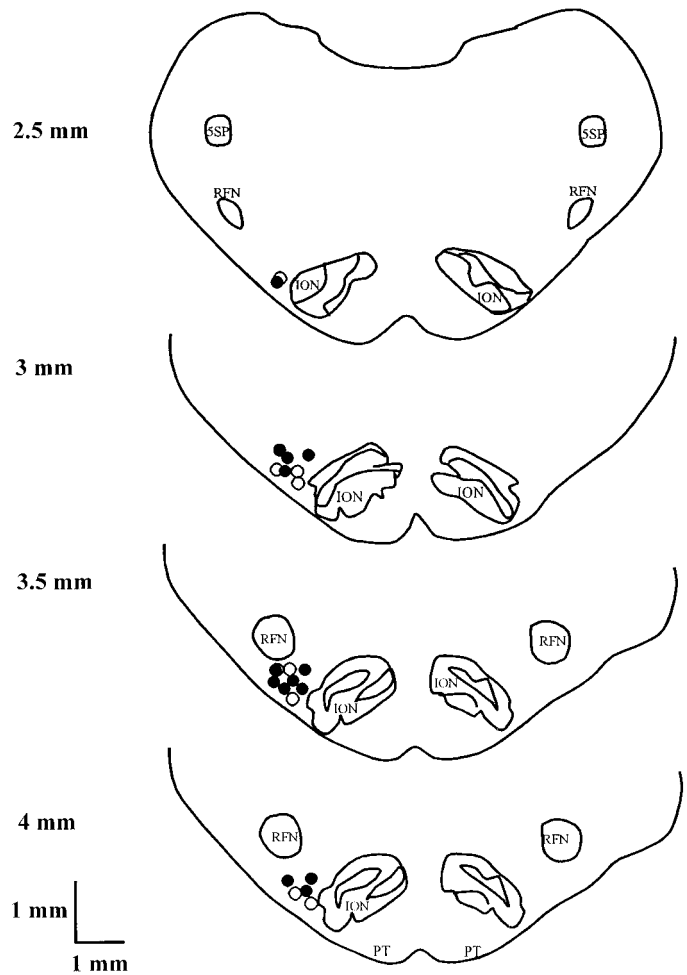


Fig. 5. Outline drawings of sections of ventral medulla illustrating sites of microinjection of naloxone. Sectional anatomy was taken from the atlas of Berman (4). Nos. indicate distance (in mm) rostral from obex; ●, naloxone injection sites; ○, control (saline) injection sites. ION, nucleus of inferior olive; RFN, retrofacial nucleus; PT, pyramidal tract; 5SP, alamina spinal trigeminal nucleus, parvocellular division.

nucleus in the hypothalamus, the ventral portion of periaqueductal gray matter, and the nucleus raphe obscurus. We postulated in the present study that activation of this system would inhibit sympathetic neurons in the PGL of the rVLM, an important center for the integration of cardiovascular reflexes (3, 36) and the source of spinal sympathetic outflow through the intermediolateral cell column (1, 23, 34). The inhibitory pathway can be activated by stimulating finely myelinated and nonmyelinated somatic afferent fibers excited by EA or acupuncture-like stimulation (18). The present results demonstrate that the reflex sympathetic response during stimulation of the gallbladder is inhibited by an opioid-related mechanism in the PGL of the rVLM during EA of Neiguan.

The application of BK to the serosal surface of the gallbladder evokes a significant, sympathetically mediated cardiovascular reflex response, as we have reported previously (18, 26, 30). Regional LV wall motion, as assessed by a single-crystal sonomicrometer (31), is increased by the BK-induced reflex response. Ligation of a small branch of the LAD supplying this region does not significantly alter resting wall motion, but reflex activation of the cardiovascular system by the application of BK to the gallbladder produced significant ischemia-induced wall motion dysfunction. The reduction of regional systolic wall thickening is the result of the augmented myocardial oxygen demand, as indicated by significant increases in BP and HR (16), associated with reflex sympathetic activation, in the setting of limited coronary blood flow (18). The resulting imbalance of the oxygen supply-to-demand ratio induces regional myocardial ischemia and, consequently, impaired regional contractile function. However, EA at the Neiguan acupoint significantly reduces myocardial ischemia and improves regional myocardial dysfunction, as indicated by the significant increase in wall thickening. This finding suggests that EA at Neiguan caused myocytes in the ischemic region to resume near-normal contractile function. Our previous study (18) indicated that this restorative effect is due to the reduction in myocardial oxygen demand secondary to inhibition of the reflex pressor response to BK, rather than an increase in coronary blood flow.

The model of sympathetically provoked ischemic dysfunction utilized in the present study is of interest because cardiac patients with limited coronary blood flow secondary to coronary atherosclerosis report angina pectoris in association with exercise or emotional stress, activities that augment sympathetic tone (35, 40). It is possible, therefore, that EA or acupuncture may be a useful therapeutic approach for the treatment of angina in some patients. In this regard, success with this approach has been reported (2, 33). Additional controlled clinical investigations are warranted to validate this potentially beneficial and inexpensive treatment modality.

We observed that intravenous injection of naloxone reversed the inhibitory effect of EA on the pressor response and resulting regional wall dysfunction produced by the application of BK on the gallbladder.

These results suggest that endogenous opioids, likely released in the CNS, mediate this inhibitory effect of EA. It has been reported that β -endorphins are present in the ventral medulla (9). Furthermore, local application of naloxone on the S area (before the root of the 12th cranial nerve and lateral to pyramid) of the ventral surface of the medulla reverses the salutary effect of EA at the Neiguan acupoint on acute ischemic myocardial injury in rabbits induced by 20-min occlusion of the LAD (13). These studies support a sympatho-inhibitory role of endogenous opioids released in the rVLM.

Opioid-receptor subtypes that contribute to the central action of EA are unknown; however, μ -, δ -, and κ -receptors each potentially could mediate this response. Other transmitters also may be involved. For instance, the improvements of LV pressure and S-T segment depression produced by EA in a rabbit model of myocardial ischemia were inhibited by microinjection of yohimbine into the rVLM (20). Thus α_2 -receptors in the rVLM also may be involved in the inhibitory effect of EA. Additional studies are necessary to identify the individual contributions of opioid-receptor subtypes and interactions with other mediators during EA of Neiguan.

Although microinjection of naloxone into the rVLM significantly reversed the effect of EA on the arterial BP response to BK (Fig. 4), it did not completely restore the response. This partial reversal may be related, at least in part, to the participation of other regions of the brain in the inhibitory pathway. It has been suggested that the nucleus arcuatus, ventral periaqueductal gray, and nucleus raphe obscurus play a role in the inhibitory effect of deep peroneal nerve stimulation on the defense reaction induced by stimulation of the hypothalamic or midbrain defense areas (17). Further studies will be necessary to determine whether these regions also are involved in the inhibitory effect of EA at the Neiguan acupoint on the gallbladder-induced pressor reflex.

The Neiguan acupoint was selected because it is one of the primary acupoints used clinically in traditional Chinese medicine to treat coronary heart disease (2, 8, 19). It also is an acupoint commonly utilized to study the effects of EA in several different animal models of cardiovascular disease (13, 18, 19). Previous preliminary studies (19) have suggested that stimulation of nerves underlying other acupoints can elicit different effects on arterial BP, but additional studies are required to address fully the issue of acupoint specificity.

There are three potential limitations of the present study. First, the use of intravenous naloxone does not allow identification of the neurological site(s) of opioid inhibition. However, this shortcoming was overcome by administering naloxone locally into a specific region of the medulla, the PGL of the rVLM. Localized injection allowed identification of the neurological locus of the opioid-mediated inhibition evoked by EA.

Second, correct positioning of acupuncture needles in patients relies on feedback from the patient to identify the onset of a feeling of "heaviness" associated with electrical stimulation of the needles when they are

properly positioned at the acupoint (43, 44). Because this information is not available in an anesthetized animal preparation, our criterion for correct transcutaneous insertion of the acupuncture needles relied on our observation of a slight repetitive flexion of the paw during electrical stimulation. In our previous study (18), in which the median nerves underlying the Neiguan acupoint were exposed and stimulated directly, the same criterion was used to establish a satisfactory level of electrical stimulation. The similar effects on BP of EA at the Neiguan acupoint in the present study, and of direct electrical stimulation of the median nerves in the previous study (18), support the postulated role of stimulation of the median nerve as the trigger event. Moreover, the similar modification of the gallbladder-induced reflex hemodynamic responses during direct stimulation of the median nerve (18) and EA of the Neiguan acupoint using a transcutaneous approach supports the general concept that the effects of acupuncture and EA are the result of stimulation of underlying nerves, particularly group III and IV somatic afferents (18, 21).

The third potential limitation is that naloxone did not completely inhibit the EA-induced effect. This could be caused by 1) insufficient naloxone and therefore insufficient blockade, 2) other areas that might still mediate the response, and 3) other potential mediators that might be important in this response.

In conclusion, the new finding in this study is that EA at the Neiguan acupoint inhibits a gallbladder-evoked pressor response by an opioid-related mechanism in the PGL of the rVLM. Naloxone administration also reversed the EA-induced improvement in regional ischemic myocardial dysfunction. The similar hemodynamic effects produced by transcutaneous EA at Neiguan and direct stimulation of median nerves support the notion that localized stimulation produced by acupuncture and EA at specific acupoints exerts an effect in the CNS by stimulation of somatic afferent nerves that, at least in some instances, underlie the acupoint along a traditional Chinese meridian.

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REFERENCES

- Amendt, K., J. Czachurski, K. Dembowski, and H. Seller. Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *J. Auton. Nerv. Syst.* 1: 103–117, 1979.
- Bao, Y. X., H. H. Lu, G. R. Yu, D. S. Zheng, B. H. Cheng, and C. C. Pan. The immediate effect on acute myocardial infarction treated by puncturing Neiguan. *Chin. Acupunct. Moxib.* 1: 2–5, 1981.
- Bauer, R. M., G. A. Iwamoto, and T. G. Waldrop. Ventrolateral medullary neurons modulate pressor reflex to muscular contraction. *Am. J. Physiol.* 257 (Regulatory Integrative Comp. Physiol. 26): R1154–R1161, 1989.
- Berman, A. L. *The Brain Stem of the Cat: A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. Madison, WI: Univ. of Wisconsin Press, 1968.
- Breyfogle, H. S. The frequency of coexisting gallbladder and coronary disease. *JAMA* 114: 1434–1437, 1940.
- Caringi, D., D. J. Mokler, D. M. Koester, and A. Ally. Rostral ventrolateral medullary opioid receptor activation modulates pressor response to muscle contraction. *Am. J. Physiol.* 274 (Heart Circ. Physiol. 43): H139–H146, 1998.
- Chao, D. M., Y. X. Cao, K. F. Pitsillides, J. C. Longhurst, and P. Li. Role of opioids in the inhibitory effect of acupuncture on the gallbladder pressor response (Abstract). *FASEB J.* 12: A690, 1998.
- Chen, S. Z. The comparative observations on the effects of puncturing Jianshi and Neiguan on the left cardiac function of patients with coronary disease. *Clin. J. Acupunct. Moxib.* 10: 30–32, 1994.
- Finley, J. C. W., P. Lindstrom, and P. Petrusz. Immunocytochemical localization of β -endorphin-containing neurons in the rat brain. *Neuroendocrinology* 33: 28–42, 1981.
- Gao, Z. W., X. Z. Yu, A. X. Shen, L. E. Bao, and X. C. Ling. Clinical observation on treatment of 220 cases of arrhythmia with acupuncture. In: *Compilation of the Abstracts of Acupuncture and Moxibustion Papers. The First World Congress on Acupuncture-Moxibustion*. Hangzhou, PRC: World Federation of Acupuncture and Moxibustion Studies, 1987, p. 13–14.
- Gatti, P. J., W. P. Norman, A. M. T. Dasilva, and R. A. Gillis. Cardiorespiratory effects produced by microinjecting L-glutamic acid into medullary nuclei associated with the ventral surface of the feline medulla. *Brain Res.* 381: 281–288, 1986.
- Holaday, J. W. Cardiovascular effects of endogenous opiate systems. *Annu. Rev. Pharmacol. Toxicol.* 23: 541–594, 1983.
- Huang, E. M., Q. Z. Feng, and P. Hu. Role of ventrolateral medullary area in the effect of electrical needling "Neiguan" point on improving acute myocardial ischemia in rabbits. *Acupunct. Res.* 16: 108–111, 1991.
- Huangfu, D. H., and P. Li. The inhibitory effect of the deep peroneal nerve input on the ventral medullary defense-reaction-related neurons. *Chin. J. Physiol. Sci.* 2: 123–131, 1986.
- Huangfu, D. H., and P. Li. The inhibitory effect of ARC-PAG-NRO system on the ventrolateral medullary neurons in the rabbit. *Chin. J. Physiol. Sci.* 4: 115–125, 1988.
- Kitamura, K., C. R. Jorgensen, F. L. Gobel, H. L. Taylor, and Y. Wang. Hemodynamic correlates of myocardial oxygen consumption during upright exercise. *J. Appl. Physiol.* 32: 516–522, 1972.
- Li, P. Modulatory effect of somatic inputs on the medullary cardiovascular neuronal function. *News Physiol. Sci.* 6: 69–72, 1991.
- Li, P., K. F. Pitsillides, S. V. Rendig, H.-L. Pan, and J. C. Longhurst. Reversal of reflex-induced myocardial ischemia by median nerve stimulation: a feline model of electroacupuncture. *Circulation* 97: 1186–1194, 1998.
- Li, P., and T. Yao. *Mechanism of the Modulatory Effect of Acupuncture on Abnormal Cardiovascular Functions*. Shanghai, PRC: Shanghai Medical Univ. Press, 1992.
- Liu, J. L., S. P. Chen, Q. S. Cao, and J. J. Zhang. Influence of microinjection of clonidine and yohimbine in rVLM on the effect of electroacupuncture treatment of myocardial ischemia. *Acupunct. Res.* 21: 31–35, 1996.
- Liu, R. T., and M. Lang. Effect of electrical needling "Neiguan" point on the promotion of the recovery of acute myocardial ischemia in cats: analysis of afferent pathways. *Acupunct. Res.* 11: 229–233, 1986.
- Longhurst, J. C. Acupuncture's beneficial effects on the cardiovascular system. *Preventive Cardiol.* 4: 21–33, 1998.
- Lovick, T. A. Descending projections from the ventrolateral medulla and cardiovascular control. *Pflügers Arch.* 404: 197–202, 1985.
- Lovick, T. A. Differential control of cardiac and vasomotor activity by neurons in nucleus paragigantocellularis lateralis in the cat. *J. Physiol. (Lond.)* 389: 23–35, 1987.
- Lovick, T. A., P. Li, and L. C. Schenberg. Modulation of the cardiovascular defense response by low frequency stimulation of

- a deep somatic nerve in rats. *J. Auton. Nerv. Syst.* 50: 347–354, 1995.
26. **Martin, S. E., D. M. Pilkington, and J. C. Longhurst.** Coronary vascular responses to chemical stimulation of abdominal visceral organs. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H735–H744, 1989.
 27. **McLemore, G. A., and S. A. Levine.** The possible therapeutic value of cholecystectomy in Adams-Stokes disease. *Am. J. Med. Sci.* 229: 396–391, 1955.
 28. **Morilak, D. A., G. Drolet, and J. Chalmers.** Cardiovascular effects of opioid antagonist naloxone in rostral ventrolateral medulla of rabbits. *Am. J. Physiol.* 258 (*Regulatory Integrative Comp. Physiol.* 27): R325–R331, 1990.
 29. **Newman, P. P.** *Visceral Afferent Functions of the Nervous System.* London: Edward Arnold, 1974, p. 35–37.
 30. **Ordway, G. A., and J. C. Longhurst.** Cardiovascular reflexes arising from the gallbladder of the cat. Effect of capsaicin, bradykinin, and distension. *Circ. Res.* 52: 26–35, 1983.
 31. **Pitsillides, K. F., and J. C. Longhurst.** An ultrasonic system for measurement of absolute myocardial thickness using a single transducer. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1358–H1367, 1995.
 32. **Punnen, S., R. Willette, A. J. Krieger, and H. N. Sapru.** Cardiovascular response to injections of enkephalin in the pressor area of the ventrolateral medulla. *Neuropharmacology* 23: 939–946, 1984.
 33. **Richter, A., J. Herlitz, and A. Hjalmarsen.** Effect of acupuncture in patients with angina pectoris. *Eur. Heart J.* 12: 175–178, 1991.
 34. **Ross, C. A., D. A. Ruggiero, A. Jon, D. H. Park, and D. J. Reiss.** Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *J. Comp. Neurol.* 228: 168–185, 1984.
 35. **Rozanski, A., C. N. Bairey, D. S. Krantz, J. Friedman, K. J. Resser, M. Morell, S. Hilton-Chalfen, L. Hestrin, J. Bietendorf, and D. S. Berman.** Mental stress and the induction of silent myocardial ischemia in patients with coronary artery disease. *N. Engl. J. Med.* 318: 1005–1012, 1988.
 36. **Stornetta, R. L., S. F. Morrison, D. A. Ruggiero, and D. J. Reiss.** Neurons of rostral ventrolateral medulla mediate somatic pressor reflex. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp. Physiol.* 25): R448–R462, 1989.
 37. **Sun, S. Y., Z. Liu, P. Li, and A. J. Ingenito.** Central effects of opioid agonists and naloxone on the blood pressure and heart rate in normotensive and hypertensive rats. *Gen. Pharmacol.* 27: 1187–1194, 1996.
 38. **Tam, K.-C., and H.-H. Yiu.** The effect of acupuncture on essential hypertension. *Am. J. Chin. Med.* 3: 369–375, 1975.
 39. **Tennant, R., and H. M. Zimmerman.** Association between disease in the gallbladder and in the heart as evidenced by autopsy. *Yale J. Biol. Med.* 3: 495–503, 1931.
 40. **Victor, R. G., D. R. Seals, and A. L. Mark.** Differential control of heart rate and sympathetic nerve activity during dynamic exercise: insight from intraneural recordings in humans. *J. Clin. Invest.* 79: 508–516, 1987.
 41. **Wakefield, H.** The association of the gallbladder disease with heart disease. *Med. Clin. North Am.* 31: 161–171, 1947.
 42. **Walker, K., M. Perkins, and A. Dray.** Kinins and kinin receptors in the nervous system. *Neurochem. Int.* 26: 1–16, 1995.
 43. **Wei-P'ing, W.** *Chinese Acupuncture.* Whitstable, UK: Straker Brothers, 1962.
 44. **Weng, E. Q.** Pain and analgesia. Shanghai, PRC: Shanghai Science and Technology, 1987, p. 182–233.
 45. **Yao, T., S. Andersson, and P. Thoren.** Long-lasting cardiovascular depression induced by acupuncture-like stimulation of the sciatic nerve in unanaesthetized spontaneously hypertensive rats. *Brain Res.* 240: 77–85, 1982.