Excitatory Projections from Arcuate nucleus to Ventrolateral Periaqueductal Gray in Electroacupuncture Inhibition of Cardiovascular Reflexes

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Abstract

We have shown that the modulatory effect of electroacupuncture (EA) on the blood pressure (BP) response induced by visceral organ stimulation is related to inhibition of cardiovascular neurons in the rostral ventrolateral medulla (rVLM) through a mechanism that involves opioids. This effect is long-lasting and may involve a long-loop neural supraspinal pathway, including the arcuate nucleus (ARC), which is an important site of opioid neurotransmitter synthesis. Therefore we evaluated the role of the hypothalamic ARC, and its interaction with the midbrain ventrolateral periaqueductal gray (vIPAG) in the EA-BP response. The gallbladder of α-chloralose anesthetized cats was stimulated to test for the influence of EA on splanchnic afferent-induced cardiovascular reflexes. Electrodes were placed around the splanchnic nerve (SN) and acupuncture needles were applied at P5-6 acupoints overlying the median nerve (MN). Electrophysiological recordings showed that spontaneous activity of ARC and vIPAG neurons was low (1.3±0.5 and 2.0±0.5 spikes/sec, respectively). We observed a gradation of responses of ARC neurons to stimulation of different acupoints, ranging from uniform responses of all neurons during stimulation of the P5-6, LI 4-11, H5-6 and St2-G2 located over deep nerves to fewer responses during stimulation of LI6-7 and G37-39 located over superficial nerves. Microinjection of the excitatory amino acid, D, L-homocysteic acid (DLH 4 nM, 50 nl), into the ARC augmented the responses of vIPAG neurons, while microinjection of kainic acid (KA 1 mM, 50 nl) to deactivate neurons in the ARC decreased vIPAG responses to SN stimulation. Thirty min of EA at P5-6 increased the SN-evoked discharge of vIPAG neurons (7.0±1.2 to 14.3±3.0 spikes/30 stimuli), a response that was blocked by microinjection of KA into the ARC. Microinjection of DLH into the ARC, like EA, inhibited (30 min) the reflex increase in BP induced by application of...
bradykinin (BK) to gallbladder, while microinjection of KA into the ARC blocked the inhibitory influence of EA at P5-6 on the BK-induced BP response. These results suggest that excitatory projections from the ARC to the vlPAG are essential to the EA inhibition of the reflex increase in BP induced by SN or gallbladder visceral afferent stimulation.
Introduction

Cardiovascular disease is the greatest cause of death in middle-aged and elderly North Americans and Europeans (32). There has been increasing interest in the western countries in exploring alternative medicinal treatments and in considering new therapies like acupuncture for cardiovascular disease. A number of small clinical reports suggest that acupuncture may reduce BP in hypertension patients (9, 21, 31). A study from our laboratory in animals showed that EA alleviates experimental reflex induced hypertension and myocardial ischemia (7). Additionally, in humans we have found that electroacupuncture (EA) can inhibit the increased blood pressure induced by dynamic exercise (19). The aim of the present study was to establish the neural activity and mechanism underlying the long-lasting effect of EA on the blood pressure response induced by splanchnic organ stimulation.

The arcuate nucleus, an important source for opioid peptides in the central nervous system (CNS) (1, 8), is thought to participate in the analgesic effect of acupuncture (28). Electrolytic lesions, electrical stimulation and recordings of evoked field potentials, as well as histochemical studies have suggested that acupuncture activates endorphin-containing neurons in the arcuate nucleus, which project to a number of other nuclei including the periaqueductal gray (PAG) (29, 30).

Others investigations (7, 12) have demonstrated that the inhibitory effect of electroacupuncture (EA) on the defense response and excitation of defense reaction-related neurons in the rVLM induced by stimulation of hypothalamic and midbrain defense areas can be abolished by electrolytic lesioning of the arcuate region. Furthermore, stimulation of the arcuate nucleus and the ventral PAG with the excitatory amino acid D, L-homocysteic acid (DLH) attenuates the defense reaction-induced blood pressure response (12, 13). Since
electrolytic lesions destroy both cell bodies and axons in passage, it is unclear if the arcuate nucleus and the PAG serve as important nuclei that process information during EA. However, prior studies have suggested the possibility of a long-loop pathway for EA inhibition that may pass through the arcuate nucleus and vPAG to the rVLM (12, 13).

Our recent work in cats (25, 26) has demonstrated that sensory input resulting from brief stimulation (<1 min) of the splanchnic nerve (as a surrogate for gallbladder stimulation) and afferent input from the Jianshi-Neiguan acupoints (P5-6) and a number of the other cardiovascularly active acupoints provide short-term excitatory input to rVLM cardiovascular premotor sympathetic neurons. Conversely, more prolonged stimulation of somatic afferents during 30 min of EA at these same acupoints inhibits excitatory cardiovascular reflex responses induced by visceral organ stimulation through a process that involves prolonged modulation of cardiovascular neurons in the rVLM. We have suggested that this inhibitory response to EA is mediated by involvement of a long-loop inhibitory neural pathway including the arcuate nucleus and the vlPAG, since our previous studies (11) showed that EA applied at the P5-6 acupoints activates neurons in the rVLM and vlPAG. Thus, in the present study, we hypothesized that, during prolonged somatic afferent stimulation, EA stimulates supramedullary pathways, including the vlPAG and the arcuate nucleus, and that activation of the arcuate nucleus, in particular, is required for the long-term EA-related cardiovascular effect. We have presented a preliminary communication of this work (18).

**Methods and Materials**

*2.1. Surgical preparation*
The experimental preparations and protocols for this study were reviewed and approved by the Animal Care and Use Committee of the University of California, Irvine CA. The studies conformed to the American Physiological Society guidelines and principles for research involving animals. Adult cats of either sex (2.1-3.6 kg) were anesthetized by injection of ketamine (40 mg/kg S.C.) followed by α-chloralose (50 mg/kg I.V.). Additional injections of α-chloralose (5 mg/kg I.V.) were given to maintain an adequate depth of anesthesia, as assessed by the lack of a response to noxious toe pinch, a respiratory pattern that followed the ventilator (i.e. not over-breathing) and a stable blood pressure. The trachea was intubated, and respiration was maintained artificially (model 66, Harvard Apparatus, South Natick, MA). Gallamine Triethiodide (4 mg/kg) was administered intravenously before recording neuronal activity to avoid any muscle movement during stimulation of somatic nerves. Following paralysis, supplemental α-chloralose was administered on a regular basis. Arterial blood gases and pH were measured periodically in all animals with a blood gas analyzer (model ABL3, Radiometer, Copenhagen, Denmark). Arterial PO$_2$ and PCO$_2$ were kept within normal limits (CO$_2$ 30-35 mmHg; PO$_2$ >100 mmHg) by enriching the inspired O$_2$ supply and adjusting the ventilation rate or volume. Arterial pH was maintained between 7.35 and 7.45 and corrected, as necessary, by administering 8% sodium bicarbonate. Body temperature was monitored with a rectal probe connected to a thermometer (model 44TD, Yellow Springs Instrument, Yellow Springs, OH) and maintained at a range of 36-38 °C by a water heating pad and a heating lamp.

The left femoral vein was cannulated for administration of drugs and fluids. Systemic arterial blood pressure was monitored by a pressure transducer (model 1290, Hewlett-Packard, Waltham, MA) attached to a cannula inserted into the left femoral artery.
A laparotomy provided exposure of the gallbladder and isolation of the splanchnic nerve. The splanchnic nerve was placed on a bipolar stimulating electrode connected to an isolation unit and stimulator (Grass, model S88). Hypoxy dental glue (Pentron, Wallington, CT) was used to isolate the electrodes and hold the intact nerve in place. The abdominal wall was closed again with clips to maintain moisture in the abdominal cavity and to prevent heat loss. Thereafter, the neural axis of the cat was stabilized with a stereotaxic head frame (Kopf). A craniotomy was performed to expose the hypothalamic arcuate nucleus and midbrain periaqueductal gray from the dorsal aspect. The abdominal cavity was reopened only when the filter paper, dipped in bradykinin (BK, 10 µg/ml), was applied to the serosal surface of the gallbladder.

2.2. Stimulation, Recording and Microinjection Methods

The splanchnic nerve was stimulated with 0.4-0.6 mA, 0.5 ms pulses at 2 Hz with a Grass stimulator (Model S88K) at a level sufficient to induce a reflex increase in blood pressure. Electroacupuncture was applied bilaterally using pulses of 1-4 mA, 0.5 ms duration and 2 Hz at the P6-5 acupoints. We demonstrated previously that EA at these locations (Neiguan and Jiangshi, respectively located 1.5-2.0 and 2.5-3.0 cm above the wrist between the ligaments of the flexor carpi radialis and the palmaris longus) stimulates the median nerve and modulates sympathoexcitatory cardiovascular responses (6, 15, 25, 26). To examine the responses of cells in the arcuate nucleus to input from different somatic nerves, stimulation also was applied bilaterally at acupoints LI6-7 (large intestine meridian), G37-39 (gallbladder meridian), K1-B67 (kidney and bladder meridians) overlying superficial radial, peroneal and branches of tibial and superficial peroneal nerves and LI4-11 (large intestine meridian), H5-7.
(heart meridian), St36-37 (stomach meridian), Sp6-9 (spleen meridian), St2-G2 (stomach-gallbladder meridians), overlying deep nerves using acupuncture needles inserted to a depth of 2-3 or 4-5 mm, respectively (26).

The arcuate nucleus was located by microinjecting D, L-homocysteic acid (DLH, 4 nM, 50 nl) to evoke a small reproducible decrease in blood pressure of 5 to 10 mmHg. Single-unit extracellular activity in the arcuate nucleus or vlPAG was recorded with a single-barrel glass pipette containing 0.5 M sodium acetate, 2% Chicago sky blue (Sigma Chemical, St Louis, MO) and a platinum recording electrode inserted 22 and 28 mm from the dorsal aspect of the midbrain and hypothalamus for the vlPAG and arcuate nucleus, respectively, according the atlas of Bureš et al. (4). Midbrain vlPAG and hypothalamic arcuate neurons were identified by the evoked excitatory neuronal responses during stimulation of the splanchnic nerve and/or P5-6 acupoints.

To evaluate the influence of EA on arcuate and vlPAG neuronal activity, action potentials were amplified with a preamplifier (Grass P511) attached to a high-impedance probe (Grass H1 P5), filtered (0.3-10 KHz) and monitored with an oscilloscope (Tectronix 2201). Action potentials, blood pressure and heart rate were digitized and analyzed online with a Pentium II computer and a four channel data acquisition system (SHMU; Shanghai Medical College of Fudan University, China). To assess the evoked response to stimulation of the splanchnic and median nerves, peristimulus time histograms were constructed for each neuron. Action potentials were analyzed both visually and with the SHMU program for similar wave shapes, heights and latency from the time of stimulation. The relationship between neuronal activity and blood pressure waves was assessed with time domain analysis using arterial pulse-triggered averaging and frequency domain analysis for coherence (25, 26).
2.3 Experimental protocols

(1) **Convergent afferent input in arcuate nucleus.** To determine the extent of convergent input in 28 arcuate nucleus neurons from visceral and somatic nerves, neuronal discharge activity was recorded during stimulation of the right splanchnic and bilateral median nerves using EA at P5-6 acupoints (30 stimuli at 2 Hz). Subsequently, LI4-11, LI6-7, H5-7, St36-37, G37-39, Sp6-9, K1-B67, St2-G2 acupoints, respectively overlying the deep radial, superficial radial, ulnar, deep peroneal, superficial peroneal, tibial and branches of tibial nerves, branches of facial and trigeminal nerves were stimulated to evaluate point-specific arcuate nucleus neuronal responses.

(2) **Effect of microinjection of DLH or KA into arcuate nucleus on vlPAG neuronal activity.** The neuronal discharge activity of 12 vlPAG neurons was recorded before and after bilateral microinjection of DLH (4 nM, 50 nl) or KA (1 mM, 50 nl) into the arcuate nucleus followed by evaluation of vlPAG neuronal responses to stimulation of splanchnic nerves or P5-6 acupoints (30 stimuli, 2 Hz).

(3) **Effect of EA at P5-6 on arcuate and vlPAG neuronal responses to splanchnic nerve stimulation.** The response of 12 arcuate neurons and 14 vlPAG neurons to splanchnic nerve stimulation was recorded before, during and after 30 min of EA. In 8 other vlPAG neurons, KA was bilaterally microinjected into the arcuate nucleus before EA during splanchnic nerve stimulation to examine involvement of the arcuate nucleus in the EA response.

(4) **Hemodynamic response.** To induce reflex increases in blood pressure, a filter paper (1 cm²) soaked in a solution of BK (10 µg/ml) was applied to the gallbladder. The blood pressure response was calculated as the difference between prestimulus mean blood pressure and pressure at the peak of the reflex response. To prevent tachyphylaxis, recovery periods of at
least 15 min were provided between consecutive stimuli. In five animals, after observing two consistent increases in blood pressure in response to application of BK on the gallbladder, DLH (4 nM, 50 nl) was microinjected bilaterally into the arcuate nucleus, followed by four applications of BK to the gallbladder at 15 min intervals to evaluate the influence of DLH on the blood pressure responses. In two other groups of animals, after observing consistent increases in blood pressure in response to BK application on gallbladder, normal saline (NS, 50 µl, n=5) or KA (1 mM, 50 µl, n=7) was microinjected bilaterally into the arcuate nucleus and the gallbladder was re-stimulated. Thereafter, EA was applied bilaterally for 30 min at P5-6 while the gallbladder was stimulated at 15 min intervals. After the completion of EA, we evaluated the magnitude of four BK–induced blood pressure responses over the next 60 min. Thus, BK was applied during control and following arcuate nucleus microinjections (three stimuli), EA (two) and recovery after EA (four), for a total of nine repeated stimuli.

2.4 Verification of injection and recording sites

At the end of each experiment, the animal was euthanized with α-chloralose followed by intravenous saturated KCL. Recording sites were marked with 2% Chicago blue dye by either iontophoresis (5 min, 400 nA) or microinjection (0.1 µl). The hypothalamus and midbrain were removed and fixed in 10% formalin for 4-7 days. Frozen serial sections (60 µm) of the brain were cut with a freezing microtome (Leica CM 1850). Slices were stained with neural red and examined with a microscope (Nikon eclipse 6400) to identify recording and stimulation sites. These sites were reconstructed from the dye spots with New Bitmap Image plotted on coronal sections separated by 2 mm with respect to the auditory line and identified using the atlas of Bureš et al. (4) as a reference.

2.5 Statistical analysis
Data are presented as means ± S.E.M. The assumption of normal data distribution was analyzed by the Kolmogorov-Smirnov test. The change in neuronal activity (evoked-baseline spikes/30 stimuli) in response to splanchnic nerve stimulation before, during and after EA, and after delivery of saline, DLH or KA were compared by a one-way repeated measures analysis of variance, followed post-hoc by the Student-Newman-Keuls test. These tests represented a pairwise multiple comparison procedure. We utilized SigmaStat and SigmaPlot software (Jandel Scientific, San Rafael, CA) for statistical analysis and graphing. The 0.05 probability level was used to determine statistically significant differences.

Results

1. **Afferent input in arcuate nucleus**

To examine the role of the arcuate nucleus in the inhibitory effect of EA, with respect to visceral and somatic afferent input, we recorded activity in 28 arcuate neurons during stimulation of the splanchnic and various somatic nerves using the stimulus paradigm employed during EA. Spontaneous discharge activity of neurons in this nucleus was 1.3±0.5 spikes/sec. There was no significant relationship between spike activity and blood pressure waves as assessed by the coherence analysis (coherence < 0.5) and arterial pulse-triggered averaging. Each neuron received convergent input from the splanchnic and one or more somatic nerves EA at specific sets of acupoints (Fig. 1). Evoked activity was presented in all 28 neurons in the arcuate nucleus during stimulation of the P5-6 acupoints. However, the response to splanchnic nerve stimulation was much smaller (4 vs 11 imp/30 stimuli) with only 21 of 28 neurons (75%) responding.
The response to stimulation of the other acupoints, including LI4-11, H5-7, St2-G2, St36-37, Sp6-9 and K1-B67 overlying the median, deep radial, ulnar, trigeminal/facial nerves, deep peroneal or tibial nerves (or a branch of these nerves) was observed to be similar to P5-6 (Fig. 1, P<0.05). On the other hand, the response to stimulation of LI 6-7 or G37-39 over the superficial radial or superficial peroneal nerves was less than the response to stimulation of P5-6.

2. Stimulation and deactivation of arcuate nucleus on vlPAG response to visceral afferent stimulation

The influence of arcuate stimulation on vlPAG activity was examined in 12 animals following bilateral microinjection of 50 nl of 4 nM DLH). Before stimulation of the arcuate nucleus with DLH, spontaneous activity in the vlPAG was 2.0±0.5 spikes/sec. Following activation of cell bodies in this region with DLH, spontaneous activity in the vlPAG increased to 4.0±0.8 spikes/sec (P<0.05). There was also a significant increase in the vlPAG response during both visceral and somatic afferent stimulation, (P<0.05; Fig. 2, Panel A). Thus, excitation of arcuate neurons increased spontaneous discharge activity and facilitated the vlPAG responses to stimulation of both visceral and selective somatic afferent pathways.

The influence of KA-induced depolarization blockade of arcuate neurons on the response of 12 other vlPAG neurons during visceral and somatic stimulation was evaluated. Bilateral blockade of the arcuate nucleus with 50 nl of 50 µM KA decreased the responses to stimulation of the splanchnic nerve and the P5-6 acupoints (P<0.05, Fig. 2, Panel B). Thus, interruption of neuronal activity in the arcuate nucleus decreased both spontaneous discharge activity and evoked responses of vlPAG neurons.

3. Influence of EA on arcuate and vlPAG response to splanchnic nerve stimulation.
In 12 arcuate neurons, we observed consistent evoked responses of 3.8±0.9 and 4.2±1.2 spikes/30 stimuli during two repeated stimuli of the splanchnic nerve (Fig. 3, Panel A). Thirty min of EA at P5-6 gradually increased activity of these neurons to 7.9±2.4 spikes/30 stimuli (P<0.05). Twenty and 30 min after cessation of EA, the evoked response remained at a higher level of activity (9.6±3.2 and 8.6±2.2 spikes/30 stimuli, respectively) and slowly returned toward control level after 60 min. Thus, EA at P5-6 facilitated the response of arcuate neurons to splanchnic nerve stimulation for a prolonged period of time.

In 14 vlPAG neurons, we observed consistent evoked responses of 7.0±1.2 and 6.9±1.4 spikes/30 stimuli during two stimulations of the splanchnic nerve. Bilateral microinjection of normal saline into the arcuate nucleus did not change the neuronal response (Fig. 3, Panel B). However, EA at P5-6 at 10, 20 and 30 min of stimulation increased the splanchnic nerve-induced activity in the vlPAG to 14±3.4, 13±2.4 and 14±3.0 spikes/30 stimuli (P<0.05, Fig. 3, Panel B; Fig. 4 A-C). The evoked response was maintained at a higher level for 30 to 40 min after cessation of EA, then slowly returned toward baseline responses. Thus, EA facilitated the vlPAG neuronal response to splanchnic nerve stimulation for more than 40 min after termination of EA. In eight other neurons outside the vlPAG (Fig. 6 C), EA did not alter the response to splanchnic nerve stimulation.

4. Response to chemical blockade of arcuate nucleus on influence of EA in vlPAG

The responses to two repeated stimulations of the splanchnic nerve in 14 vlPAG neurons averaged 4.3±1.4 and 3.9±1.1 spikes/30 stimuli. Bilateral microinjection of 50 nl of 50 µM KA into the arcuate nucleus, eliminated the EA-related augmentation of activity in the vlPAG during splanchnic nerve stimulation (P>0.05; Fig. 3, Panel C; Fig. 4 D-F). In other five animals when KA was injected to areas outside the ARC we observed no alteration of the EA
augmentation of vlPAG activity in response to splanchnic nerve stimulation (Fig. 6 B). Thus, chemical blockade of cellular activity specifically in the arcuate nucleus prevented the facilitatory effect of EA in vlPAG neurons.

5.  **Influence of arcuate nucleus on hemodynamic response to EA during gallbladder stimulation**

Repeated BK stimulation of gallbladder chemosensitive afferents evoked consistent increases in blood pressure of 37-43 mmHg (P>0.05) for more than two hours (Fig. 5, Panel A). Bilateral microinjection of 50 nl of 4 nM DLH into the arcuate nucleus attenuated the hemodynamic response by 44%, an effect that lasted for 30 min before returning to control (Fig. 5C). Resting blood pressure before each reflex stimulation was constant throughout the protocol (P>0.05).

The influence of EA following injection of NS into the arcuate nucleus reduced the blood pressure response to 54% of control values (Fig. 5, Panel B). This inhibition continued for 45 min, gradually subsiding 60 min after cessation of EA. In this group of animals, resting blood pressure before each reflex response was not significantly different throughout the protocol (P>0.05) and was similar to the baseline blood pressure in the control group (Fig. 5, Panel A).

We observed that bilateral microinjection of kainic acid in the arcuate nucleus delayed the inhibitory influence of EA for 45 min after EA termination (Fig. 5, Panel D). Resting blood pressure in this group was not altered by either EA or KA (P>0.05).

6.  **Anatomic location of recording and microinjection sites.**

Injection sites for all of the protocols were shown to be localized to the arcuate nucleus or vlPAG (Fig. 6). Injections either dorsal or caudal to the arcuate nucleus did not influence the BK-induced increase in blood pressure (Fig. 6, Panel A) or the responses of vlPAG neurons to
Eight neurons recorded outside the vIPAG, (dorsal, lateral or ventral) displayed either an inhibitory or no effect during EA (Fig. 6, Panel D and E).

**Discussion**

We have demonstrated previously that the inhibitory effect of EA on the blood pressure response induced by visceral organ stimulation is related to the modulation of cardiovascular neurons in the rVLM (6, 15). This inhibition is long-lasting, averaging 45-60 min after termination of EA. We have suggested that the prolonged influence of EA involves a long-loop supra-spinal pathway (25). The present study demonstrates that arcuate nucleus serves as an important center in this long-loop neural pathway.

Neurons in the arcuate nucleus are rich in endorphins and enkephalins (1) and may be essential for the analgesic effect attributed to acupuncture (28, 29, 30). Our electrophysiological studies demonstrate that afferent input from stimulation of the splanchnic nerve and a number of different acupoints located over somatic neural pathways converge onto common arcuate neurons. We observed that, while only 75% of neurons in the arcuate nucleus examined receive input from the splanchnic nerve, 100% of neurons in this region receive input from the median nerve (underlying P5-6), deep radial nerve (underlying LI4-11), and ulnar nerve (underlying H6-7). Their responses to somatic stimulation also were much higher than splanchnic nerve evoked activity. The trigeminal and facial nerves (St2-G2) and pathways in the lower limbs, including the deep peroneal (St36-37) and tibial nerves (Sp6-9), provided similar input to the arcuate as P5-6. Conversely, the LI 6-7 (overlying superficial radial nerve) and G37-39 (overlying superficial peroneal nerves) supply less or no input to this region. The extent of convergent input from the different somatic nerves underlying
acupoints used by acupuncturists supports the inclusion of these acupoints in the treatment of cardiovascular disease involving altered blood pressure. Consistent with our earlier study on the role of the rVLM in the EA response (25), input to the arcuate nucleus was minimal from superficial radial and peroneal nerves. The LI 6-7 and G37-39 acupoints potentially can be used as control acupoints in clinical acupuncture studies (19).

Our data show that arcuate neurons are activated by EA and that the prolonged response to splanchnic nerve stimulation remains at a high level for at least 40 min after cessation of EA. Also, neuronal discharge in the vlPAG was influenced by neuronal activity in the arcuate nucleus. Thus, both spontaneous activity and the responses of the vlPAG to stimulation of the splanchnic or median nerves underlying the P5-6 acupoints were facilitated by excitation or suppressed following deactivation of cell bodies in the arcuate nucleus. Results of previous studies on the relationship between the arcuate nucleus and vlPAG are conflicting. For example, Strahlendort et al. (24) reported inhibition of vlPAG neurons by electrical stimulation of arcuate nucleus. However, Huangfu and Li (12, 13) using microinjection of the excitatory amino acid, DLH, into the arcuate nucleus observed excitatory responses of ventral PAG neurons. Differences in experimental techniques may explain the discrepancies between these studies. In this respect, electrical stimulation excites both axons in passage and cell bodies, while chemical stimulation excites only cell bodies. In the present study, we postulated that projections from neurons in the arcuate nucleus to the vlPAG would be predominately excitatory, a hypothesis that was confirmed by using both stimulation and deactivation of the neurons in the arcuate nucleus.

Different regions in the PAG in the midbrain are thought to exert a differential influence on cardiovascular function (3). For example, stimulation of the dorsal PAG
produces an excitatory response including tachycardia and vasodilatation of the hindlimb, characteristic of the cardiovascular defense reaction (20). Conversely, the vlPAG mediates decreases in blood pressure (5, 14). The present study shows that excitatory projections from the arcuate nucleus were confined to the vlPAG, since misplacement of electrodes (lateral, dorsal or ventral to the vlPAG) did not record an excitatory response.

In addition to observing excitatory projections from the arcuate nucleus to the vlPAG, we observed excitation of the vlPAG during stimulation of the splanchnic nerve, a response that was facilitated by EA. This facilitatory effect appeared soon after the onset and terminated slowly after the cessation of EA. The EA-related facilitation in the vlPAG was abolished by chemical deactivation of the neurons in the arcuate nucleus. These data thus provide evidence for an important interaction between the arcuate nucleus and the vlPAG in the EA response. The long-lasting influence may partially explain the prolonged inhibitory effect of EA on sympathoexcitatory cardiovascular neurons in the rVLM (15, 17, 25). Future studies will need to focus on the neurotransmitter mechanisms underlying the interaction between the arcuate nucleus and the vlPAG, between the vlPAG and the rVLM and between neurons in the arcuate nucleus and the rVLM as well as the neurotransmitters related to long-lasting inhibition of rVLM neurons by EA.

In the present study, like EA, microinjection of DLH into the arcuate nucleus inhibited the blood pressure response induced by application of BK on the gallbladder, while depolarization blockade of the arcuate nucleus by KA prevented the EA-related inhibition. As such, while excitation of arcuate nucleus, as a surrogate for EA, can inhibit the blood pressure response, blockade of neuronal activity in the arcuate nucleus eliminates the inhibitory effect of EA on the excitatory cardiovascular reflex. Thus, these hemodynamic data provide
additional data confirming the important role of the arcuate nucleus and the vlPAG in the EA-related inhibition of excitatory cardiovascular reflex responses.

We recently have begun to explore relationship between the vlPAG and the rVLM (27). Our data show that there is an inhibitory projection from the vlPAG to the rVLM. Thus, excitation of neurons in the arcuate nucleus and the vlPAG ultimately may lead to inhibition of activity in sympathoexcitatory bulbo-spinal neurons in the rVLM. The three nuclei together appear to form part or the entire long-loop pathway that seems critical for the EA-related modulation of reflex excitatory hemodynamic responses.

In sum, our results suggest that the slow onset and long-lasting effect of EA on reflex increase in blood pressure may be related to the activation of neurons in the arcuate and vlPAG nuclei, and to inhibition of rVLM premotor sympathetic cardiovascular neurons.

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Figure Legends

Fig. 1. Convergent input in arcuate neurons. Bar histograms show consistent convergent input to arcuate neurons from points P5-6 as well as the LI 4-11, H5-7, St 36-37, Sp6-9, St2-G2, K1-B67, overlying the deep radial, ulnar, deep peroneal, tibial, branches of facial and trigeminal, branches of tibial nerves. However, responses from LI6-7 and G37-39 acupoints overlying superficial radial and superficial peroneal nerves were much less than that observed during P5-6 stimulation. Numbers on the top of each column are means ± SE of each response. Below each figure are listed the number responses compared to number of somatic nerves stimulated. * Indicates increased discharge rate compared to response during stimulation of P5-6 (P< 0.05).

Fig. 2. Activity of vlPAG neurons in response to stimulation or inactivation of arcuate nucleus by D, L-homocysteic acid (DLH) and kainic acid (KA), respectively. Microinjection of DLH into the arcuate of 12 cats increased evoked responses to splanchnic nerve and P5-6 stimulation (Panel A, n=12). In contrast, microinjection of KA into arcuate nucleus in 12 other cats inhibited evoked responses during splanchnic nerve and P5-6 stimulation (Panel B, n=12). * Indicates different discharge frequencies compared to control response before DLH or KA (P< 0.05).
Fig. 3. Excitatory projections from the arcuate nucleus to the vlPAG during EA. EA at P5-6 facilitated the neuronal response to splanchnic nerve stimulation in the arcuate and vlPAG nuclei in 14 studies (Panel A and B, respectively). Microinjection of KA into the arcuate nucleus in 8 other cats prevented the excitatory influence of EA in the vlPAG (Panel C). * Indicates increased (P<0.05) responses compared to controls. Abbreviation: NS- normal saline. 10 min- time interval between bars (for all panels).

Fig. 4. Facilitatory effect of EA at P5-6 on the response of vlPAG neurons to splanchnic nerve stimulation. Panels A and D provide peristimulus histograms of two vlPAG neurons showing their excitatory response to splanchnic nerve stimulation (0.4 mA, 2 Hz, 0.5 ms pulse duration) before EA. Panel B: increase the change of neuronal response during 30 min EA at P5-6 after normal saline (NS) injection into the arcuate nucleus (ARC). Panel F: no increase of neuronal response during 30 min EA at P5-6 after KA injection into arcuate nucleus. Panel C and F: recovery of neuronal response 50 min after termination of EA. Insert in panel A, displays neurogram of vlPAG neuron responding to stimulation of the splanchnic nerves.

Fig. 5. Role of the arcuate nucleus in cardiovascular blood pressure response before and after EA. Panel (A) histograms showing consistent increase in blood pressure in respect to repeated application of BK on gallbladder every 15 min in five cats. Second panel: original recordings of blood pressure from an individual animal of hemodynamic changes induced by application of BK to gallbladder before microinjection of normal saline (NS) (a), after EA for 30 min (b) and 50 min after termination of EA (c). Panel B: effect of microinjection of NS into the arcuate nucleus before 30 min of EA at P5-6 during repeated reflex responses in five
cats. Panel C: influence of microinjection of DLH into arcuate nucleus on the cardiovascular blood pressure responses in five cats. Panel D: response to microinjection of KA into the arcuate nucleus on the cardiovascular inhibitory EA response in seven animals. * Indicates significant changes (P<0.05) compared to control before intervention. Means ±SEM beneath each bar represent resting blood pressure prior to each stimulus.

Fig. 6. Composite map of brain sections showing locations of microinjection, stimulation and recording sites in arcuate nucleus and vIPAG. Panel A: microinjection sites in arcuate nucleus (ARC) during hemodynamic studies. For simplification all the injections of KA are displayed on the right side of each section, injections of NS or DLH on the left. Panel B: microinjection sites in arcuate nucleus during electrophysiological studies. The injections of KA are shown on right and injections of NS are on left. Panel C: recording sites in arcuate nucleus on left and microinjection of DLH on right. Panel D and E: recording sites in vIPAG. Symbols: ♦: KA to ARC; ◊: KA outside ARC; ■: Saline to ARC; □: Saline outside ARC; +: microinjection of DLH in ARC; -: microinjection of DLH outside ARC; *: recording in ARC; x: Recording outside ARC; ●: Recording in vIPAG. o: Recording outside vIPAG.

Spikes/30 stimuli

Splan  P5-6  LI4-11  H5-7   St36-  Sp6-9   K1-   Si2-G2   LI6-7  G37-39

n= 21/28   28/28   13/13    7/7    12/14    6/7    6/7    11/12      4/6      2/4

* 4+1  11+2  14+4  7+3  11+5  6+4  6+2  2+1  2+2

-------------Deep nerves-------------

Cranial nerves  24±8

Superficial nerves

Fig. 1
A.

![Graph showing viPAG spikes/30 stimuli for Control and DLH-ARC groups. The graph indicates a significant increase in the DLH-ARC group compared to the Control group. The asterisk (*) denotes statistical significance.](image)

B.

![Graph showing viPAG spikes/30 stimuli for Control and after KA groups. The graph indicates a significant increase in the after KA group compared to the Control group, with asterisks (*) denoting statistical significance.](image)

Fig. 2
Fig. 3

A.

ARC
Spikes/30 stimuli

0
5
10
15
20

EA P5-6

B.

NS

vPAG
Spikes/30 stimuli

0
5
10
15
20

EA P5-6

C.

KA-ARC

vPAG
Spikes/30 stimuli

0
5
10
15
20

EA P5-6

10 min
Fig. 4
Fig. 5