Brain stem mechanisms underlying acupuncture modality-related modulation of cardiovascular responses in rats

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Submitted 8 December 2004; accepted in final form 30 March 2005

Zhou, Wei (Yi Syuu), Stephanie C. Tjen-A-Looi, and John C. Longhurst. Brain stem mechanisms underlying acupuncture modality-related modulation of cardiovascular responses in rats. J Appl Physiol 99: 851–860, 2005. First published April 7, 2005; doi:10.1152/japplphysiol.01365.2004.—The present study was designed to investigate brain stem responses to manual acupuncture (MA) and electroacupuncture (EA) at different frequencies at pericardial P (5–6) acupoints located over the median nerve. Activity of premotor sympathetic cardiovascular neurons in the rostral ventral lateral medulla (rVLM) was recorded during stimulation of visceral and somatic afferents in ventilated anesthetized rats. We stimulated either the splanchnic nerve at 2 Hz (0.1–0.4 mA, 0.5 ms) or the median nerve for 30 s at 2, 10, 20, 40, or 100 Hz using EA (0.3–0.5 mA, 0.5 ms) or at ~2 Hz with MA. Twelve of 18 cells responsive to splanchnic and median nerve stimulation could be antidromically driven from the intermediolateral columns of the thoracic spinal cord, T2–T4, indicating that they were premotor sympathetic neurons. All 18 neurons received baroreceptor input, providing evidence of their cardiovascular sympathoexcitatory function. Evoked responses during stimulation of the splanchnic nerve were inhibited by 49 ± 6% (n = 7) with EA and by 46 ± 4% (n = 6) with MA, indicating that the extent of inhibitory effects of the two modalities were similar. Inhibition lasted for 20 min after termination of C fibers, significantly increases blood pressure (BP), heart rate (HR), and myocardial contractility (25, 26). Distension in the rat’s stomach likewise increases BP (21, 48) and thus provides an appropriate model involving stimulation of mechanoreceptors to study the influence of acupuncture on the cardiovascular responses to reflex activation. Additionally, during ischemia and inflammation, a number of chemical mediators, such as kinins, prostaglandins, serotonin, histamine, and reactive oxygen species, among others, initiate strong reflex sympathoexcitatory responses (27). The afferent pathways mainly follow splanchnic nerves and, to a lesser extent, the vagus nerves. The efferent pathways of this reflex include cardiac and visceral sympathetic outflow (27). Such reflex responses are significantly reduced when low-current and low-frequency electroacupuncture (EA; 0.3–0.5 mA, 2 Hz) stimulation is applied at P 5–6 acupoints in rats (21).

Physiological cardiovascular responses to acupuncture depend on the technique and stimulation parameters employed during its application. For example, the type of stimulus modality [manual acupuncture (MA) or EA] or stimulation frequency can influence the experimental results (4, 9, 17, 23, 48). In this respect, we have demonstrated recently that EA or MA at low (2 Hz) but not EA at mid (40 Hz) or high (100 Hz) frequency significantly inhibits reflex excitatory cardiovascular responses induced by visceral afferent stimulation in rats (48). This study demonstrated that low-frequency electrical and mechanical stimulation of the median nerve similarly activated Aδ- and C-fiber somatic afferents, indicating that sensory neural pathways provide important input to the central nervous system during both forms of acupuncture. A limitation of our laboratory’s previous study (48) was the interference by the stimulation artifact, which obscured action potentials from single-unit recordings used to identify the neural fiber type activated during acupuncture particularly at frequencies above 20 Hz. Furthermore, the central neural responses to different acupuncture frequencies or different modalities (e.g., EA and MA) were not determined. In this respect, since input into the central nervous system during both MA and different frequencies of EA is polysynaptic and may be influenced by both short- and long-loop pathways involving the hypothalamus and midbrain, each of which may process the input, assessment of the fidelity of response to acupuncture in brain stem regions at the terminal portion of the loop that directly regulate sympathetic premotor and hence cardiovascular activity is important.

One particularly relevant area in the brain stem that receives input from both visceral and somatic afferent systems is the rostral ventral lateral medulla (rVLM) (44, 45). Not only is there substantial information confirming the importance of this area

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area as a principal source of descending sympathetic premotor projection from the brain stem to the spinal cord (8) but also our laboratory’s prior studies have shown that this region serves as an important brain stem site for processing information during EA through mechanisms that involve both opioid and nociceptin neurotransmitter modulatory systems (5, 7, 20, 21). As such, there is a strong rationale for investigating the response of rVLM neurons, including those that are cardiovascular sympathoexcitatory in nature, during variation of stimuli that regulate input from somatic afferents activated during acupuncture.

Therefore, the general aim of this study was to investigate rVLM neuronal processing of input during stimulation at P 5–6 using modalities (MA vs. EA) and stimulation frequencies relevant to clinical practice in acupuncture. We hypothesized that MA and EA similarly inhibit visceral reflex input in the rVLM and that the extent of medullary neuronal activation, like the responses of somatic afferents, is dependent on stimulation frequency, with a higher fidelity of response occurring with low- vs. high-frequency EA.

METHODS

Surgical Procedures

Experimental preparations and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. 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, as assessed by the lack of response to noxious toe pinch and the ability to artificially maintain a consistent respiratory rate. The right jugular vein was cannulated for administration of sodium bicarbonate and α-chloralose. The trachea was intubated, and artificial respiration was monitored with a ventilator (model 661, Harvard Apparatus). The right or left carotid artery was cannulated and attached to a pressure transducer (Statham P23 ID, model 661, Harvard Apparatus). The right jugular vein was cannulated for administration of nitroglycerin or phenylephrine was evaluated. Fi-

Additionally, the influence of altered baroreceptor input following the splanchnic or median nerves. Neurons were also examined for convergence from the median (EA at P 5–6) and splanchnic nerves. Neurons were also examined for convergence from the median (EA at P 5–6) and splanchnic nerves. Additionally, the influence of altered baroreceptor input following administration of nitroglycerin or phenylephrine was evaluated. Fina-

Stimulation and Recording Methods

The splanchnic nerve was stimulated with pulses of 0.2–0.5 mA and 0.5-ms duration at 2 Hz, which was sufficient to induce a reflex pressor response. EA was applied bilaterally at P 5–6 acupoints using 0.3–0.5 mA, 0.5-ms duration at 2 Hz. Two acupoints are required for stimulation using the positive and negative poles of the electrode so that current does not pass through the body of the animal (21). The P 6 acupoint (Neiguan) was located ~3 mm proximal to the flexor crease on the forepaw over the median nerve. The P 5 acupoint (Jianshi) was located ~2 mm central to P 6 over the median nerve. The IML was stimulated electrically (0.1–0.4 mA, 0.5-ms duration, 2 Hz) to evoke a reproducible increase in BP of 15–30 mmHg to confirm placement of the stimulating electrode in the IML for collision testing. Stimuli were delivered to the IML while the extracellular recording electrode was advanced slowly through the rVLM. Single-unit activity in the rVLM was recorded with a glass pipette containing 0.5 M sodium acetate, 2% Chicago blue (Sigma, St. Louis, MO), and a platinum recording electrode. Action potentials were amplified and filtered (0.3–10 kHz) with a preamplifier (Grass P511) and monitored with an oscilloscope (Tektronix 2201). Action potentials, BP, and HR were digitized and analyzed offline with a Pentium III computer and Enhanced Graphics Acquisition and Analysis (EGAA) software (RC Electronics).

Axonal conduction velocities of rVLM neurons were calculated by dividing the distance between the thoracic IML and the recording site in the rVLM by the latency of conduction of the antidromic response from the IML to the rVLM. To assess evoked responses to stimulation of the splanchnic and median nerves, peristimulus time histograms were constructed for each response. Action potentials were analyzed for similar wave shapes, heights, and latencies from the time of stimulation with the EGAA program. The relationship between neuronal activity and BP waves was assessed by both time and frequency domain analysis using arterial pulse-triggered averaging and coherence analysis (44, 45).

Experimental Protocols

Experimental protocol diagram is shown in Fig. 1.

Protocol 1: evoked activity in rVLM cells. To search for responsive neurons in the rVLM, the IML was stimulated continuously at 2 Hz, while the recording electrode was lowered slowly at increments of 1 μm through the rVLM. Neurons in the rVLM that responded to stimulation of the IML were tested for criteria that indicated antidromic activation. Initially, they were examined for constant latency, a stable threshold of the evoked all-or-none response, and a faithful response to high rates of stimulation (200 Hz). The refractory period was used to establish the critical interval (the latency plus the refractory period) to ensure collision (24). Neurons then were evaluated for evidence of collision of triggered antidromic spikes from the IML with evoked orthodromic action potentials induced by stimulating the splanchnic or median nerves. Neurons were also examined for convergence from the median (EA at P 5–6) and splanchnic nerves. Additionally, the influence of altered baroreceptor input following administration of nitroglycerin or phenylephrine was evaluated. Finally, the discharge pattern of the 18 rVLM neurons was evaluated with respect to cardiovascular rhythmicity using time and frequency domain analysis (3, 18, 44, 45).

Spontaneous and evoked rVLM responses during stimulation of the splanchnic and median nerves were recorded. Then, the rVLM neuronal discharge patterns in response to repeated stimulation of the splanchnic nerves (afferent supply of the stomach) were recorded in five rats every 10 min for 110 min to establish baseline variability. Other preparations were processed for evidence of collision of triggered antidromic spikes from the IML with evoked orthodromic action potentials induced by stimulating the splanchnic or median nerves. Neurons were also examined for convergence from the median (EA at P 5–6) and splanchnic nerves. Additionally, the influence of altered baroreceptor input following administration of nitroglycerin or phenylephrine was evaluated. Finally, the discharge pattern of the 18 rVLM neurons was evaluated with respect to cardiovascular rhythmicity using time and frequency domain analysis (3, 18, 44, 45).
after acupuncture stimulation. The 13 animals were divided into two subgroups (MA and EA). For the MA group, acupuncture needles were inserted bilaterally into P 6 acupoints and were twisted manually at a frequency of ~2 Hz for 2 min in six rats. We repeated the 2-Hz, 2-min needle stimulation every 10 min for 30 min. Alternatively, needles were inserted bilaterally at P 5–6 and were stimulated electrically at 2 Hz and 0.3–0.5 mA for 2 min every 10 min in seven other rats. The immediate interaction during simultaneous brief stimulation (30–40 s, 0.3–0.5 mA, 0.5-ms duration, 2 Hz) of the splanchnic and the median nerves (at P 5–6) in seven cardiovascular neurons in the rVLM was evaluated. All seven rats demonstrated occlusive interaction. Subsequently, neuronal activity in the rVLM evoked by the stimulation of the splanchnic nerve every 10 min was recorded for up to 60 min after termination of EA or MA to determine the response to acupuncture and the time course of recovery of neuronal activity.

Protocol 3: frequency-related rVLM neuronal activity. Our laboratory’s previous study showed that low-frequency (2 Hz) electrical stimulation activates many more somatic afferents than high-frequency stimulation (48). In the present study, we evaluated the response of rVLM neurons to electrical stimulation of somatic afferents at different frequencies to determine whether there was concordance between the peripheral and central neuronal responses. We also recorded the activity of eight premotor sympathetic cardiovascular rVLM neurons during 30–40 s of electrical stimulation (0.3–0.5 mA, 0.5-ms duration) at P 5–6 at 2, 10, 20, 40, or 100 Hz, in random order. The interval between each period of stimulation was 5 min.

Histology

Recording sites were marked by microinjection of 0.1 μl of 2% Chicago blue dye through the barrel of the electrode. The brain was removed and fixed in 10% paraformaldehyde for 4–7 days. Brain stems were sliced with a microtome cryostat at a thickness of 40 μm. Recording sites were reconstructed from dye spots with the aid of a microscope (Nikon) and software (Corel presentation). The rVLM was defined as the region lateral to the inferior olivary nucleus and pyramidal tract and both ventral and medial to the facial and retrofacial nuclei. It includes the medial and caudal parts of nucleus paragigantocellularis (35). These sites are located within the rVLM as defined previously by others (3, 12). Data were excluded if recorded neurons were not located in the area of rVLM, as defined above.

Data Analysis

Data are presented as means ± SE. Neuronal activity (impulses/30 stimulations) in response to splanchnic nerve stimulation before, during, and after EA or MA was compared by analysis of variance and post hoc by the Student-Newman-Keuls test. Differences (control vs. EA or MA; MA vs. EA) were compared with the Student’s t-test. All statistical analyses were performed with the software package Sigma Stat (Jandel Scientific). The 0.05 probability level was used to determine statistically significant differences.

Time and frequency relationships between rVLM activity and arterial BP were evaluated using coherence as well as pulse-triggered spike analysis. Coherence was determined with the fast Fourier transform algorithm (34). Original data were recorded with a sampling rate of 10,000 Hz; reconstructed data utilized every 10th sample. Reconstructed data included assessment of the mean and peak amplitudes as well as the maximum and minimum slopes of the original spike to be certain that all action potentials were preserved. Cells were subjected to spike height discrimination before coherence analysis. Auto spectra of rVLM discharge and arterial BP were generated using fast Fourier transform. Coherence was developed with seven overlapping windows each with the same length (12.8 s) consisting of 256 bins with a bin width of 50 ms. The autospectral analysis was adopted from Shin et al. (38) using contiguous segments of 256 beats with 50% overlap between contiguous segments. The frequency resolution was 1/12 s or 0.08 Hz. The coherence function (normalized cross-spectrum) measures the strength of linear correlation of two signals at each frequency. Coherence values of ≥0.5 were chosen to reflect a statistically significant relationship between rVLM spikes and arterial BP (3, 18, 39, 41). The time relationship of rVLM activity with arterial BP was evaluated using pulse-triggered analysis with a threshold that was set at the systolic phase of the arterial pulse. Averages of the arterial pulse and histograms of the neuronal activity were constructed for analysis (3, 18, 44, 45).

RESULTS

Protocol 1: Evoked Activity in rVLM Cells

We studied 18 neurons with baseline activity of 3.2 ± 0.4 impulses/s. Twelve neurons could be driven antidromically
from the IML of the spinal cord. Figure 2A displays an example of the collision test used to define, in part, rVLM neurons that were stimulated antidromically and, hence, could be classified as premotor sympathetic. The antidromically induced spike collided with the action potential evoked by stimulation of the splanchnic nerve. The distance from IML to rVLM was 22 mm, onset latency 7.5 ms, and calculated conduction velocity 2.9 m/s. Overall, the distance between the IML and the rVLM was 23 ± 2.2 mm, the onset latency was 7.3 ± 1.4 ms, and the calculated axonal conduction velocity was 3.1 ± 1.2 m/s (n = 12).

All 18 neurons were examined by several methods with respect to their relationship to cardiovascular-related activity. Discharge frequencies of these neurons were subjected to frequency and time domain analysis, coherence, as well as arterial pulse-triggered averaging to evaluate their relationship to the cardiovascular rhythm. We found a strong correlation (average coherence value 0.81 ± 0.06) of neuronal activity with arterial BP at a frequency of 6.2 ± 0.4 Hz. Arterial pulse-triggered averaging likewise demonstrated a strong relationship between the discharge rate of the 12 antidromically driven neurons and arterial BP. Figure 2B shows the arterial pulse-triggered analysis. A neuron with a coherence of 0.9 at a frequency of 6.4 Hz is shown in Fig. 2C.

We also examined the response of rVLM neurons to baroreceptor input using nitroglycerin or phenylephrine to alter BP, depending on baseline activity. Figure 2D displays the response of a neuron to nitroglycerin. This neuron’s activity was increased from 3.6 to 14 impulses/s by nitroglycerin. Nitroglycerin increased discharge activity of 14 neurons from 3.3 ± 0.7 to 15 ± 4.1 impulses/s, whereas phenylephrine decreased activity consistently in each of the other 5 neurons from 3.7 ± 0.7 to 0.7 ± 0.5 impulses/s.
0.8 to 2.2 ± 0.5 impulses/s, indicating that the cells were barosensitive.

**Protocol 2: Effect of MA or EA at P 5–6 on Activity of rVLM Neurons**

The immediate peak responses of rVLM neurons evoked by 30 s of either manual or electrical stimulation at P 5–6 at 2 Hz were similar (MS: 3.8 ± 0.6 vs. ES: 4.6 ± 0.8 impulses/s, 5 neurons each; Fig. 3A). The activity of the rVLM neurons evoked by brief stimulation (30–40 s, 0.3–0.5 mA) of the splanchnic and median nerves was 28 ± 4 and 36 ± 7 impulses/30 stimulations, respectively. These activities were decreased significantly to 19 ± 3 impulses/30 stimulations during simultaneous stimulation of both nerves (Fig. 3B; n = 7), thus demonstrating an occlusive interaction. An example of convergent input from the splanchnic and median nerves in an rVLM neuron is shown in Fig. 3C. In 5 of the 18 neurons, the evoked firing rate of 29 ± 6.3 impulses/30 stimulations was verified to be consistent during repeated stimulation of the splanchnic nerve every 10 min over a period of 110 min in the absence of acupuncture stimulation (Fig. 4A).

When the median nerve was stimulated with EA (Fig. 4B) and MA (Fig. 4C) at P 5–6, which were matched for frequency and duration of application, we observed a similar immediate and prolonged reduction of rVLM neuronal activity evoked by splanchnic nerve stimulation that lasted 50 min in both cases. The evoked response to stimulation of the splanchnic nerve was inhibited by 49% (29 ± 9 to 15 ± 5 impulses/30 stimulations; n = 7) with EA and by 46% (25 ± 9 to 14 ± 4 impulses/30 stimulations; n = 6) with MA, suggesting that the extent of inhibition by the two modalities was similar.

**Protocol 3: Frequency-Related rVLM Neural Activity**

We observed different responses in sympathetic premotor rVLM neuronal activity during EA at 2, 10, 20, 40, and 100 Hz (Fig. 5). There was a reciprocal relationship between the frequency of stimulation and the average rVLM neuronal response (53 ± 10.0 vs. 16 ± 2.4, 7.7 ± 1.7, 2.0 ± 1.1, and 0 ± 0 impulses/30 stimulations; Fig. 5A). Figure 5, B–F, shows the activity of a sympathetic premotor rVLM neuron responding to 2-Hz EA and to a lesser extent to EA at 10, 20, 40, and 100 Hz.

**Histology**

Examination of the brain slices revealed that all recording sites were within the rVLM as described previously (35, 40). Neuronal activity was recorded in an area that was 1.3–3.0 mm caudal to interaural line, 1.6–2.3 mm lateral to the midline, 0.2–1.0 mm from the ventral surface, lateral to the inferior olive nucleus and the pyramidal tracts, as well as ventral and medial to the facial and retrofacial nuclei (Fig. 6). Sites of stimulation also were identified to be in the IML, located in the central lateral gray area of the spinal cord between T2 and T4, a location similar to a previous report by Sun et al. (40) and Paxinos and Watson (35).

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**Fig. 3.** Histograms representing group data of rVLM neuronal discharge activity before and during 30 s of either manual (n = 5) or electrical (n = 5) stimulation of the median nerve at P 5–6 (A) as well as group data (n = 7) of sympathetic premotor rVLM neuronal responses to stimulation of splanchnic and median nerves (B). Electrical stimulation of each pathway evoked 31 and 42 responses, respectively (C; left and middle). There was an immediate occlusive interaction during simultaneous stimulation of both nerves (C; right). The neuron was classified as cardiovascular sympathoexcitatory since it was inhibited by baroreceptor input and demonstrated collision during stimulation of the IML of the spinal cord. *P < 0.05: MA and EA responses vs. spontaneous activity. †P < 0.05: median nerve or SN evoke responses vs. median + SN responses.
DISCUSSION

We previously showed that somatic afferents stimulated with low-frequency electrical or mechanical manipulation of acupuncture needles significantly inhibits reflex excitatory cardiovascular responses induced by gastric distension through stimulation of Aδ- and C-fiber somatic afferents (47, 48). Therefore, sensory neural input constitutes an important mechanism underlying the acupuncture-related cardiovascular regulation. Even though brain stem activation by sensory neuronal signals elicited during EA might be assumed based on our previous study, the fidelity of the response and the extent of processing data at a brain stem level that directly regulates sympathetic outflow (i.e., in sympathetic premotoneurons) is unclear. The present study overcomes limitations of the previous study. We clearly demonstrated that the rVLM faithfully transmits somatic information during both EA and MA. Furthermore, we noted a strong response to 2-Hz somatic afferent stimulation but increasingly smaller responses in the rVLM as the frequencies are increased from 10 to 100 Hz. This finding supports and extends the tentative conclusions derived from our laboratory’s previous somatic afferent recordings (47, 48). Importantly, demonstration in the present study that there is a high fidelity of transmission of information indicates that there is not substantial processing of peripheral signals (action potentials) before they reach the region that directly regulates sympathetic outflow from the IML of the spinal cord.

There is mounting evidence to suggest that neurons in the rVLM integrate information in a number of different conditions, including sensorimotor (afferent-effector organ)-related activation during cardiovascular regulation (28). In the present study, we examined cardiovascular neurons in the rVLM, which received both visceral and somatic input. We characterized many of the neurons using four criteria. First, they were activated antidromically during stimulation of the IML in the spinal cord to demonstrate that they were premotor sympathetic neurons. The mean conduction velocity of axons projecting from the rVLM to the thoracic IML was 3.1 ± 1.2 m/s, similar to other studies that have identified premotor sympathetic neurons in rats (39, 41). Second, these neurons either were inhibited during activation of the baroreceptor reflex by intravenous injection of phenylephrine or were excited by intravenous administration of nitroglycerin, indicating that these cells were sympathoexcitatory cardiovascular neurons. Third, coherence analysis demonstrated a significant relationship between the discharge of rVLM neurons and arterial BP at frequencies of 5.0–6.5 Hz, which is within the range of baseline HR in these animals. Fourth, arterial pulse-triggered analysis showed that discharge activity of these neurons was related closely to the cardiac rhythm. These properties are consistent with premotor sympathoexcitatory cardiovascular neurons that have been characterized previously (3, 12, 39, 41). Additionally, neurons examined in this study received convergent input from visceral and somatic afferents.

Recently, our laboratory showed that both MA and EA have a similar inhibitory influence on the sympathoexcitatory cardiovascular response to gastric distension when frequency and duration of stimulation are matched (47, 48). Low-frequency electrical and mechanical stimulation of the median nerves...
similarly activate Aδ- and C-fiber somatic afferents, suggesting that the extent of sensory neural pathway activation, in part, determines the influence of acupuncture. However, the central neuronal response to MA and EA influences is unknown. The present study examined rVLM neuronal responses evoked with two different acupuncture modalities, MA and EA at the P 5–6 acupoints, underlying the median nerves on the distal forelegs of rats. As noted above, neurons in the rVLM play a role in central integration of a number of circulatory reflexes (29). In particular, the rVLM plays a crucial role in regulating premotor sympathetic outflow to spinal cord and ultimately the cardiovascular system (8). Our group recently demonstrated that rVLM premotor sympathetic cardiovascular neurons receive convergent input from the gallbladder through the splanchnic nerve and Jianshi-Neiguan (P 5–6) acupoints overlying the median nerves of cats (44). Electrical stimulation of P 5–6 acupoints caused an immediate excitatory response in the rVLM, and simultaneous stimulation of the splanchnic and median nerves produced an occlusive response (44). In the present study involving rats, short-term (30 s) manual or electrical stimulation of Neiguan acupoints likewise caused an immediate excitatory response in the rVLM, and we noted an occlusive response during simultaneous stimulation of the splanchnic and median nerves. Thus our observations in rats are consistent with those of our laboratory’s previous study in cats (44).

The immediate occlusive response observed during simultaneous visceral and somatic afferent stimulation likely is not the mechanism underlying acupuncture-related inhibition of sympathetic outflow. Both MA and EA were associated with prolonged inhibition of rVLM neuronal activity. The decrease in neuronal activity paralleled the prolonged inhibition of the cardiovascular reflex responses during long-term (30 min) somatic afferent stimulation associated with either MA or EA (47, 48). Our laboratory’s prior work has shown that EA attenuates cardiovascular pressor responses by inhibiting the premotor sympathetic cardiovascular neurons in the rVLM through both opioid and nociceptin systems (5, 7, 20, 44). Inhibition of cardiovascular sympathoexcitatory reflex responses during EA involves a frequency-dependent activation of somatic nerves (47, 48). Stimulation at low (2 Hz), but not mid (40 Hz) or high (100 Hz), frequencies inhibits the cardiovascular pressor reflex induced by gastric distension. Low-frequency (2 Hz) electrical stimulation activates many more somatic afferents than high-frequency (10 or 20 Hz) stimulation. A limitation of our previous study was the interference created by the stimulation artifact, which may have obscured recording single-unit action potentials used to identify the...
frequencies of EA have reached different conclusions. For mechanism in the rVLM underlying the acupuncture-related frequency-dependent somatic afferent input likely serves as one processing occurring during low-frequency input. This fre- thentic neurons and hence the greatest opportunity for signal during EA with the strongest input to rVLM premotor sympa- there is frequency-dependent activation of somatic afferents. Thus, conclusive data from both studies strongly suggest that any appreciable afferent or rVLM neuronal responses at frequencies above 20 Hz. We cannot exclude the possibility that other afferent nerves subserving other acupoints or other regions of the brain, involved with cardiovascular or pain regulation, such as the arcuate nucleus and the periaqueductal gray, may respond differently than the rVLM. However, we predict that because there is so little, if any, afferent input at middle or high frequencies of EA, examination of other potentially relevant centers involved with processing nociceptive stimuli will lead to similar results, as we have observed in the rVLM.

In addition to the rVLM, a number of supraspinal centers, including the arcuate nucleus, periaqueductal gray as well as the nucleus raphe obscurus, in the hypothalamus, midbrain, and medulla, may each play a role in the influence of EA on the cardiovascular reflex excitation (15, 16, 22, 46). Also, descending pathways from the rVLM to the spinal cord may influence segmental processing of somatic inputs during EA. In fact, there is reason to expect that a portion of the EA response may occur at the spinal level, since there are opioid systems (involving both dynorphin and enkephalin) in the dorsal horn that are governed by descending inputs (13, 36, 37). Furthermore, since the clinical practice of acupuncture has been shown to be most effective when it is practiced through a segmental approach (30, 32), these spinal systems are likely components in its central neural mechanism of action. However, the present study focused on supraspinal processing in the rVLM since this region is a critical part of central nervous system integration during EA. In this respect, the cardiovascular influence of EA can be virtually eliminated by neurotransmitter receptor blockade of opioid or nociceptin systems in this region (5, 7, 20, 21). Both spinal and supraspinal centers that receive and process somatic afferent input during EA deserve further study in the future.

In conclusion, the present study demonstrates that rVLM premotor sympathetic cardiovascular neurons that receive convergent input from the splanchnic and median nerves during low-frequency EA and MA are inhibited similarly for prolonged periods by low-frequency MA and EA. Thus, when matched for frequency and time of stimulation, both forms of stimulation have comparable capabilities in regulating sympathetic outflow. We have also found that sympathetic premotor rVLM neurons demonstrate a high-fidelity response to low- but not high-frequency median nerve stimulation, an observation that is parallel to our previous study of the frequency response of somatic afferents during EA at P 5–6 (48). Activation of sympathetic afferents, such as the splanchnic nerve, for example during food ingestion (21, 25, 26) or acute and chronic inflammatory diseases like cholelithiasis and cholecystitis (27), by augmenting the myocardial ischemia, has the potential to evoke excitatory cardiovascular reflexes, including hypertensive responses and tachyarrhythmias, which lead to significant morbidity and mortality (31, 33). The present study suggests that either MA or EA, particularly at low frequencies, has the

Fig. 6. Composite map displaying sites of neuronal recordings (*) in rVLM. All 18 neurons received convergent input from somatic and splanchnic nerves. Sections are 1.3–2.0, 2.0–2.5, and 2.5–3.0 mm caudal to the interaural line [Paxinos and Watson, 1999 (35)]. LPGi, lateral paragigantocellular nucleus; Py, pyramidal tract; Amb, ambiguous nucleus; 7, facial nucleus; Sp5, spinal trigeminal nucleus; IO, inferior olivary nucleus; MVe, SuVe, and LvVe, medial, superior, and lateral vestibular nuclei, respectively; Sol, solitary tract nucleus; 4V, 4th ventricle. rVLM is an area within the LPGi. The demarcated area indicates the rVLM area taken from Paxinos and Watson Rat Brain Atlas, which outlines the LPGi/rVLM region.

response and the neural fiber type activated during higher frequency (40 or 100 Hz) stimulation (48). However, from our laboratory’s previous investigation (48), we suggest that there would be negligible input with high frequencies because the afferent response was significantly reduced between 2 and 20 Hz. In the present study, we observed a similar frequency-dependent drop off in response of rVLM neurons. Particularly, we observed strong responses to 2-Hz somatic afferent stimulation but increasingly smaller rVLM responses as the frequencies were increased from 10 to 100 Hz. This is consistent with our tentative conclusion from our laboratory’s previous study. Thus, conclusive data from both studies strongly suggest that there is frequency-dependent activation of somatic afferents during EA with the strongest input to rVLM premotor sympa- thetic neurons and hence the greatest opportunity for signal processing occurring during low-frequency input. This fre- quency-dependent somatic afferent input likely serves as one mechanism in the rVLM underlying the acupuncture-related inhibition of cardiovascular excitatory responses.

Interestingly, some studies examining responses to different frequencies of EA have reached different conclusions. For instance, it has been claimed that acupuncture analgesia can be induced by either low-frequency (2–4 Hz) or high-frequency stimulation (100 or 200 Hz) (10, 11, 14). These studies have focused on the mechanisms of EA-induced analgesia and have suggested that stimulation with 2-Hz EA mobilizes enkephalin, whereas 100 Hz releases dynorphin (10, 11, 14). However, we question the conclusions of these previous studies since neither our previous nor present study have demonstrated any appreciable afferent or rVLM neuronal responses at frequencies above 20 Hz. We cannot exclude the possibility that other afferent nerves subserving other acupoints or other regions of the brain, involved with cardiovascular or pain regulation, such as the arcuate nucleus and the periaqueductal gray, may respond differently than the rVLM. However, we predict that because there is so little, if any, afferent input at middle or high frequencies of EA, examination of other potentially relevant centers involved with processing nociceptive stimuli will lead to similar results, as we have observed in the rVLM.

In conclusion, the present study demonstrates that rVLM premotor sympathetic cardiovascular neurons that receive convergent input from the splanchnic and median nerves during low-frequency EA and MA are inhibited similarly for prolonged periods by low-frequency MA and EA. Thus, when matched for frequency and time of stimulation, both forms of stimulation have comparable capabilities in regulating sympathetic outflow. We have also found that sympathetic premotor rVLM neurons demonstrate a high-fidelity response to low- but not high-frequency median nerve stimulation, an observation that is parallel to our previous study of the frequency response of somatic afferents during EA at P 5–6 (48). Activation of sympathetic afferents, such as the splanchnic nerve, for example during food ingestion (21, 25, 26) or acute and chronic inflammatory diseases like cholelithiasis and cholecystitis (27), by augmenting the myocardial ischemia, has the potential to evoke excitatory cardiovascular reflexes, including hypertensive responses and tachyarrhythmias, which lead to significant morbidity and mortality (31, 33). The present study suggests that either MA or EA, particularly at low frequencies, has the
potentially improve these cardiovascular conditions (5, 19, 21, 48). Thus the present study provides a better understanding of interactions between visceral and somatic afferents input to the rVLM, underlying the action of specific forms of acupuncture in regulation of cardiovascular function. These data may have direct clinical implications for the practice of EA or MA.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Vu Thai Nguyen for technical assistance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-63313 and HL-72125, and the Larry K. Dodge Endowed Chair (J. C. Longhurst).

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