Medullary Substrate and Differential Cardiovascular Responses in response to Specific Acupoints

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ABSTRACT

Electroacupuncture (EA) at P5-P6 acupoints overlying the median nerve reduces premotor sympathetic cardiovascular neuronal activity in the rostral ventral lateral medulla (rVLM) and visceral reflex pressor responses. In previous studies, we have noted different durations of influence of EA comparing P5-P6 and S36-S37 acupoints, suggesting that point specificity may exist. The purpose of this study was to evaluate the influence of stimulating P5-P6, LI4-L7, LI6-LI7, LI10-LI11, S36-S37 or K1-B67 specific acupoints, overlying deep and superficial somatic nerves, on the excitatory cardiovascular reflex and rVLM responses evoked by stimulation of chemosensitive receptors in the cat’s gallbladder with bradykinin (BK) or direct splanchnic nerve (SN) stimulation. We observed point specific differences in magnitude and duration of EA-inhibition between P5-P6 or LI10-LI11 and LI4-L7 or S36-S37 in responses to 30 min stimulation with low frequency, low current EA. EA at LI6-LI7 and K1-B67 acupoints as well as direct stimulation of the superficial radial nerve did not cause any cardiovascular or rVLM neuronal effects. Cardiovascular neurons in the rVLM, a subset of which were classified as premotor sympathetic cells, responded to brief (30 s) stimulation of the SN as well as acupoints P5-P6, LI10-LI11, LI4-L7, S36-S37, LI6-LI7 or K1-B67, or underlying somatic pathways in a fashion similar to the reflex responses. In fact, we observed a significant linear relationship ($r^2=0.71$) between the evoked rVLM response and reflex change in mean arterial blood pressure. In addition, EA stimulation at P5-P6 and LI4-L7 decreased rVLM neuronal activity by 41 and 12 %, respectively, for over an hour demonstrating that prolonged input into the medulla during stimulation of somatic nerves, depending upon the degree of convergence, leads to more or less inhibition of activity of these cardiovascular neurons. Thus, EA at acupoints overlying deep and superficial somatic nerves leads to point specific effects on cardiovascular reflex responses. In a similar manner, sympathetic cardiovascular rVLM neurons that respond to both visceral (reflex) and somatic (EA) nerve stimulation manifest graded responses during stimulation of specific acupoints suggesting that this medullary region plays a role in site-specific inhibition of cardiovascular reflex responses by acupuncture.

Keywords: somatic afferents, electroacupuncture, visceral afferents, rostral ventral lateral medulla, prolonged neuronal inhibition
INTRODUCTION

Alternative medicine is used with increasing frequency in the United States and most other countries. In particular, manual acupuncture and the more potent alternative, electroacupuncture (EA), are used primarily for treatment of chronic ailments including cardiovascular disease. Chinese and European physicians have suggested that EA is effective in alleviating certain cardiovascular diseases and their symptoms, including hypertension, arrhythmias and angina (4,14,19,26,48,49,56). One well recognized set of acupoints, Jianshi-Neiguan (located along the pericardial meridian, P5-P6), is positioned directly over the median nerve on the wrist, and is used frequently to treat symptomatic coronary heart disease (14,26,37). Our laboratory has developed a feline model of a partial coronary artery occlusion to study the mechanism of EA’s cardiovascular influence during stimulation of the P5-P6 acupoints as well as the median nerve directly. Our work has demonstrated that low current (13) (2-4 mA), low frequency (5 Hz) EA stimulation of the P5-P6 acupoints in cats significantly reduces the extent of myocardial ischemia brought about by an imbalance between oxygen supply and demand during reflex increases in arterial blood pressure, caused by the stimulation of chemosensitive sensory nerve endings in the gallbladder (12,32). The EA effect appears to be caused mainly (~ 67%) by stimulation of the Aδ-fibers in the median nerves, which, in part, activate opioid receptors in the rostral ventrolateral medulla (rVLM), that inhibit sympathetic outflow and the resulting cardiovascular pressor responses induced by application of bradykinin (BK) on the gallbladder (12). We also have shown that the order of potency of opioid receptors for EA-inhibition is $\mu=\delta>>k$, suggesting that endorphins, endomorphin and enkephalins are the main neuromodulators in this process (33). Our most recent studies have demonstrated that some cardiovascular premotor sympathetic rVLM neurons receive input from both the gallbladder (splanchnic) and median nerves. Through an opioid mechanism, many of these neurons display prolonged decreases in activity for more than 50 min after 30 min of EA at P5-P6 (57).

The acupoints P5-P6, Hegu-Lique (along the lung meridian, LI4-L7, overlying the superficial radial and median nerves), Shousanli-Quchi (along the large intestine meridian, LI10-LI11, over the deep radial nerves) and Zusanli-Shangjuxu (along the stomach meridian, S36-S37, over the deep peroneal nerves) have been used by acupuncture practitioners to treat not only cardiovascular conditions (4,16,19), but also nausea, gastrointestinal diseases (14) and pain (55). Experimental studies have confirmed the efficacy of several of these acupoints. For instance, stimulation of the median nerve below the P5-P6 acupoints reduces a visceral pressor reflex (12,33). Furthermore, stimulation of the deep peroneal nerve beneath the S36-S37 acupoints reduces the frequency of ventricular extrasystoles induced by stimulation.
of the hypothalamus (21,22,61) as well as a visceral pressor reflex (35). However, little is known about the relative efficacy of these acupoints and their influence on rVLM neurons.

The present study therefore examined the modulatory influence of EA on the whole animal pressor reflex and on activity of medullary neurons during selective stimulation of specific sets of acupoints overlying somatic nerves, several of which are thought to be useful in treatment of cardiovascular disease (14). Several evidence-based studies (34,50,59) define acupoint specificity as the cardiovascular response to stimulation of different acupoints in pathologically or physiologically altered conditions. We concentrated on the rVLM since this is one region that integrates the influence of EA on premotor sympathetic outflow and hence cardiovascular function (12,33). We hypothesized that the influence of EA is similar in its action on medullary activity and on the integrated physiological hemodynamic output. Thus, we examined cardiovascular point specific effects of six sets of acupoints overlying the median (P5-P6), superficial (LI6-LI7) and deep radial (LI10-LI11), deep peroneal (S36-S37), terminal branch of tibial nerves (K1-B67) or branches of the median nerve and the superficial radial nerve (LI4-L7) (2,27,52) with respect to: 1) the gallbladder-pressor reflex and 2) evoked activation of medullary cardiovascular sympathetic neurons in cats.

Nomenclature, locations of acupoints and nerves beneath acupoints, indicated in references like Standardized Acupuncture Nomenclature and Chinese-English Dictionary of Acupuncture, are tabulated to provide clarity (Table 1) (1,2,27,51,52).

MATERIALS AND METHODS

Surgical Procedures

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of the University of California, Irvine. Studies were performed on cats of either sex (2.5-4.5 kg). Animals were anesthetized initially with ketamine (40 mg/kg, im). The left femoral vein was cannulated to enable administration of alpha-chloralose (50 mg/kg) and other drugs. Supplemental doses of alpha-chloralose (10 mg/kg) were given as necessary to maintain an adequate depth of anesthesia, as assessed by the animals’ lack of response to noxious toe pinch, a respiratory pattern that followed the ventilator and a stable blood pressure (BP). Cats were intubated and artificially ventilated (model 661, Harvard Apparatus). Arterial blood gases and pH were measured every hour with a blood gas analyzer (ABL5, Radiometer America) and were maintained within the normal range (PCO₂ 32-35 mmHg;
PO_2 >100 mmHg) by enriching the inspired O_2 supply and adjusting the ventilatory rate or volume. Arterial pH was kept between 7.35-7.40 and was corrected as necessary by administration of sodium bicarbonate. Body temperature was monitored with a rectal probe (model 44TD) and was maintained between 36-37.5°C by a thermostatically controlled heating pad and a heating lamp. Systemic BP was measured with a cannula inserted into the femoral artery, which was connected to a pressure transducer (model 1290, Hewlett-Packard).

A laparotomy enabled exposure of the gallbladder and isolation of the splanchnic nerve. The isolated 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 maintain isolation and to hold the nerve in place. The abdominal wall was closed 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 spinal holder and a stereotaxic head frame (Kopf). A laminectomy and a craniotomy were performed to expose the spinal cord (T_1-T_3) and the dorsal medulla, respectively. The abdominal cavity was reopened only when BK was applied to the serosal surface of the gallbladder.

In some protocols, somatic nerves were isolated and stimulated directly to ensure neural activation. Thus, following isolation, bipolar stimulating electrodes were placed around the median, deep radial or superficial radial nerves to examine for convergent input into rVLM neurons and to evaluate the influence of 30 min of stimulation of the superficial radial nerves on pressor responses induced by stimulation of the gallbladder.

Stimulating electrodes were positioned below the dorsolateral sulcus at a depth of 0.8-1.5 mm to reach the intermediolateral column (IML) between the T_2 and T_4. The electrodes were lowered in increments of 50 μm into the spinal cord searching for the lowest stimulus threshold required to induce a pressor response. At the end of the experiment, the site was marked with an electrolytic lesion and verified histologically. Recording electrodes were positioned perpendicularly to the dorsal surface of the medulla, 2.75-3.0 mm lateral to the midline and 2.75-3.0 mm rostral to the obex and were lowered 5.0 mm into the medulla to reach the rVLM. Recording sites were marked with Chicago blue dye at the end of each experiment.

**Stimulation and Recording Methods**

The splanchnic nerve was stimulated with 0.4-0.6 mA, 0.5 ms duration, 2 Hz. Electroacupuncture was applied bilaterally at the P5-P6 acupoints using 4 mA, 0.5 ms duration at 2-4 Hz (13,57). We have confirmed previously that EA at this location stimulates the median nerve and modulates sympathoexcitatory cardiovascular responses (12,32). The thoracic spinal cord was stimulated electrically (0.1-0.4 mA, 2 Hz, 0.5 ms duration) or chemically (D, L-homocysteic acid, 4 nM, 50 nl) to
evoke a small but reproducible pressor response of 5-10 mmHg, which along with anatomical location after the experiment, confirmed the location of the stimulating electrode for collision testing in the IML. Then, stimuli were delivered to the IML while an extracellular recording electrode was advanced slowly through the rVLM. Single-unit activity in the rVLM was recorded with a platinum electrode inserted in the barrel of a glass pipette filled with 0.5 M sodium acetate containing 2% Chicago sky blue (Sigma Chemical, St Louis, MO). Action potentials were amplified with a preamplifier (Grass P511) attached to a high-impedance probe (Grass H1 P5) and were filtered (0.3-10 kHz) and monitored with an oscilloscope (Tektronix 2201). Action potentials, BP and heart rate were digitized and were analyzed offline with a Pentium III computer and EGAA software (R. C. Electronics).

Conduction velocities of rVLM neurons were measured by stimulating the thoracic IML, recording in the rVLM and estimating the distance between the two sites. To assess the evoked response to stimulation of splanchnic and median nerves, peri stimulus time histograms were constructed for each neuron. Action potentials were analyzed both visually and with the EGAA program for similar wave shapes, heights and latency from the time of stimulation. The relationship between neuronal activity and blood pressure waves was assessed by both time and frequency domain analysis using arterial pulse-triggered averaging and coherence analysis (7,57).

Electroacupuncture was applied bilaterally at the P5-P6, LI4-L7, LI 6-LI7, LI10-LI11, S36-S37 or K1-B67 acupoints (1,2,51,52). Needles were placed at a depth of ~ 4 mm (deep somatic nerves) or ~ 1 mm (superficial nerves). An electrical stimulator with an isolation unit provided current to acupuncture needles, one located at each acupoint (two for the set of two acupuncture points, e.g., P5-P6) to deliver bipolar stimulation for either brief (30 s) or more prolonged periods (30 min). Brief stimulation of the somatic nerves was used to examine the immediate responsiveness of these neurons. Prolonged stimulation was used during EA, to simulate the clinical use of this procedure. During direct somatic nerve stimulation (0.4-0.6 mA, 0.5 ms duration, 2-4 Hz), two electrodes were placed bilaterally around the nerves. Each electrode was separated by 5 to 7 mm and connected to the stimulator to deliver bipolar stimulation in a manner similar to the stimulation procedure with percutaneous acupuncture needles.

**Experimental Protocols**

**Effects of EA on pressor reflexes**

To induce pressor responses, chemosensitive afferents of the gallbladder were stimulated with a one cm² pledget of filter paper soaked with a solution of BK (10 µg/ml). After the maximum pressor reflex was attained, the filter paper was removed and the gallbladder was washed four times with normal saline to remove BK. The pressor response was calculated as the difference between prestimulus mean blood pressure to pressure at the peak of the reflex response. To prevent tachyphylaxis, recovery periods
of at least 15 min were provided between applications (43,46). In seven groups of animals included in the hemodynamic studies, pledgets soaked with BK were used to induce pressor responses (25-50 mmHg). After observing two consistent increases in blood pressure in response to stimulation with BK, we began bilateral EA at specific acupoints using acupuncture needles inserted percutaneously or direct somatic nerve simulation, seven to ten min before the third application of BK; stimulation was continued for 30 min. During EA, BK was applied two times at 15 min intervals. After the completion of EA, BK was applied every 15 min for the next 60 to 75 min. Thus, BK was applied during control (two responses), EA (two responses) and recovery following EA (four or five responses) for a total of eight or nine data points. In another group of control animals, we tested repeatability of nine sequential pressor responses induced by BK applied to the gallbladder every 15 min in the absence of EA or direct stimulation of somatic nerves.

*Somatic afferent input to rVLM cells*

Thirty-seven cells were recorded and 39 protocols were conducted. Seventeen cells were used to examine convergent input, while 22 neurons were studied with respect to their long-term inhibitory responses. Twenty three of the 37 cells were identified as premotor sympathetic neurons.

We identified responsive neurons in the rVLM using one of two paradigms. The first method was to locate neurons that could be antidromically driven from the IML. Using this method in 23 neurons, 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 evaluated for criteria that indicated antidromic activation. First, 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). Second, the neurons then were evaluated for evidence of collision of triggered antidromic spikes from the IML with either spontaneous or stimulus-induced orthodromic action potentials evoked by stimulating the splanchnic or median nerves. The antidromically driven neurons were examined for faithful responses when the time interval between the stimulus and the evoked action potential was greater than the sum of the latency and the refractory period. Collision was observed when the sum of the latency and the refractory period was greater than the time interval (57). Conduction velocities of neurons in the rVLM that projected to the IML were determined by measuring the distance between the thoracic IML and the recording site in the rVLM and dividing this number by the latency of conduction of the antidromic response from the IML to the rVLM (57). Neurons were examined for convergent input from the median nerve during stimulation at acupoints P5-P6 and splanchnic nerve. Stimuli were applied at 2 Hz. We measured evoked neuronal activity over a 15 s period to construct the peri-stimulus histograms (57). We also recorded baseline activity of these
neurons to determine if their firing patterns exhibited a cardiac rhythmicity (57). Secondly, in 14 other rVLM neurons, after recording spontaneous activity to determine their cardiac rhythmicity (57), the neurons were categorized by recording their evoked responses during stimulation of the splanchnic nerve and the P5-P6 acupoint, using peri-stimulus histograms (57).

After the cells were identified, they were examined for either convergence or prolonged inhibition. We evaluated convergent input from different nerves during a 30 s period of stimulation of acupoints or their underlying nerves. The prolonged inhibitory response to EA was used to evaluate point specific responses.

To determine the extent of convergence in each of the 17 of the 37 neurons, evoked activity during brief (30 s) stimulation of the splanchnic, median, deep radial and/or superficial radial nerves, and/or stimulation at the acupoints: P5-P6, LI4-L7, LI10-L11, LI6-L17, S36-S37 or K1-B67, were recorded. Some of these neurons were tested for chemosensitive afferent input with application of bradykinin on the gallbladder. Thus, responses of all neurons were examined during stimulation of splanchnic nerve and P5-P6 acupoints as well as several other acupoints and/or their underlying nerves. The only exception to the practice of confirming the response to percutaneous acupuncture by direct nerve stimulation was for the Yongquan-Zhiyin acupoint (K1-B67), overlying the terminal tibial nerve, which was composed of diffuse nerve fibers rather than a discrete nerve bundle that could be isolated and directly stimulated. Additionally, to characterize further the ‘cardiovascular’ responsiveness of rVLM neurons, the effect of altered baroreceptor input following administration of nitroglycerin or phenylephrine was evaluated. Finally, for all 17 rVLM neurons, the firing pattern was evaluated with respect to cardiovascular rhythmicity using arterial pulse-triggered averaging and coherence analysis.

To evaluate the extent of prolonged inhibition lasting for ≥ one hr, we evaluated the response of 22 rVLM neurons identified as cardiovascular premotor sympathetic or barosensitive cells during splanchnic nerve stimulation every 10 min for a 30 s period before, during and after 30 min of EA at three sets of acupoints. Of note, our previous investigation (57) demonstrated that most rVLM cells (86%) receiving input from splanchnic and median nerves function as premotor sympathetic neurons. We examined the responses to EA at acupoints P5-P6, LI4-L7 and LI6-LI7 in six, five and five neurons, respectively. In addition, discharge activity during splanchnic nerve stimulation without EA was recorded every ten min in six neurons as a time control group.

Histology

At the end of each experiment, the animal was euthanized with alpha-chloralose and saturated KCl. The brain stem then was removed and fixed in 10% formalin. Frozen serial sections (60 µm) of the brain and spinal cord were cut with a freezing microtome (Leica CM 1850). Slices were examined with
a microscope (Nikon eclipse 6400) to identify the recording and stimulation sites. Recording sites were reconstructed with Corel Presentation and plotted on coronal sections separated by less than 2 mm intervals with respect to the obex (10). Stimulating sites of the spinal cord IML also were confirmed with the aid of a microscope.

**Data analysis**

Data are presented as means ± SE. The assumption of normal data distribution was analyzed by the Kolmogorov-Smirnov test. BP responses to BK were analyzed with a one-way repeated measures analysis of variance, followed post-hoc with the Student-Newman-Keuls test. These tests represented a pair wise multiple comparison procedure. We utilized SigmaStat and SigmaPlot software (Jandel Scientific, San Rafael, CA) for statistical analysis and graphing. The level of statistical significance was chosen as P<0.05.

The efficacy of responses was examined by comparing the percentage change of increase of mean arterial blood pressure between the second and forth or fifth application of BK (i.e., 30-45 min after onset of acupuncture). We defined the duration of the EA-induced decrease in the visceral reflex pressor response as the time from the initial decrease after onset of EA to the time when the EA-related decrease in each individual animal was equivalent to or greater than the smallest, but statistically significant, decrease for the overall group. Thus, if the smallest EA-induced decrease in the reflex response for a group was 10 mmHg, then EA-related changes for each animal had to be equivalent to or greater than the number to be considered significant. This procedure allowed us to assess each animal independently rather than simply using the group means to determine the duration of effect.

The levels of convergence on medullary neurons were analyzed by a one-way repeated measures analysis of variance and post-hoc with the Student-Newman-Keuls to compare the blood pressure responses to direct stimulation of splanchnic, median, superficial radial and deep radial nerves, as well as stimulation at P5-P6, LI10-LI11, LI4-L7, S36-S37, LI6-LI7 and K1-B67 acupoints. Comparison of the EA-related modulation of the reflex pressor response (change in MAP) with EA-evoked excitatory activity of the rVLM neurons was evaluated by linear regression analysis.

The extent of prolonged inhibition of rVLM neurons also was analyzed by a one-way repeated measures analysis of variance and post-hoc with the Dunn’s test to evaluate the decrease in activity in response to EA. The decrease in activity during and after acupuncture was defined as percentage decrease determined by comparing the areas under the time-neuronal activity curve for P5-P6, LI4-L7 relative to the time control.

We also evaluated time and frequency relationships between rVLM activity and arterial blood pressure using pulse-triggered spike analysis as well as coherence. Arterial pulse-triggered analysis used
a threshold that was set at the systolic phase of the arterial pulse. We used spike height discrimination to sort action potentials during the 300 s period of evaluation. Averages of the arterial pulse and histograms of neuronal activity then were constructed as described by Barman and Gebber (5).

Coherence between rVLM activities and arterial blood pressure was determined with a Fast Fourier Transform (FFT) algorithm (47, 57). Original data were recorded using a sampling rate of 10,000 Hz; reconstructed data utilized every tenth sample and 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. The cells were subjected to spike height discrimination prior to coherence analysis. Autospectra of rVLM discharge and arterial blood pressure were generated with the FFT. Coherence was generated with seven overlapping windows, each with a length of 12.8 s, consisting of 256 bins and bin widths of 50 ms. The auto-spectral analysis was adopted from Shin et. al., 1995 (52) 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) provided a measure of the strength of linear correlation of rVLM neuronal activity and blood pressure at each frequency. Coherence values of ≥0.5 were chosen to reflect a statistically significant relationship between rVLM spikes and arterial blood pressure (7,30,53,57).

RESULTS

**Acupuncture Point Specific Influence of EA on Visceral Pressor Reflex Responses**

During repeated stimulation of chemosensitive sensory nerve endings in the gallbladder every 15 min, in the control group of seven animals, we observed consistent pressor responses (Fig. 1A). The repeatable pressor responses remained between 41±6 and 49±5 mmHg for at least two hours and fifteen min. Baseline heart rate and mean blood pressure before stimulation of each reflex was unchanged throughout the protocol.

Separate application of EA at each of four sets of acupoints including P5-P6, LI10-LI11, LI4-L7 and S36-S37 inhibited the sympathoexcitatory pressor responses but to different degrees. Thus, after two consistent increases in blood pressures induced by application of BK on the gallbladder in each of the four groups comprising 29 animals overall, we observed that EA at each of the four sites modulated the reflex pressor responses to gallbladder stimulation by 42, 39, 25 and 32%, respectively (Fig. 1B-E). Acupuncture at two other sets of acupoints, LI6-LI7 and K1-B67, did not influence the reflex pressor response (Fig. 1, F and H). Likewise, direct stimulation of the superficial radial nerves underneath LI6-LI7 did not alter the reflex response (Fig. 1G).
The extent of modulation of the reflex pressor response was significantly greater with acupoints P5-P6 and LI10-LI11 compared to LI6-LI7, K1-B67 or direct stimulation of the superficial radial nerve (Fig. 2A). In contrast, the degree of attenuation caused by stimulation at P5-P6, LI10-LI11, LI4-L7 and S36-S37 was not significantly different.

The duration of the prolonged EA inhibitory effect on the visceral pressor reflex was observed in four different sets of acupoints. We observed that inhibition by EA at acupoints P5-P6, LI10-LI11, LI4-L7, and S36-S37 lasted for 72±10, 60±12, 26±5 and 24±6 min, respectively (Fig. 1B-E and Fig. 2). Stimulation at P5-P6 and LI10-LI11 modulated the pressor reflex significantly longer than stimulation at LI4-L7 or S36-S37 (Fig. 2B).

**Evoked activity in rVLM Cells**

The rVLM neurons evaluated represent selected cells identified with specific criteria to define their responses to visceral-somatic-cardiovascular convergent input. Only 60% of the cells that received median nerve input also were evoked by stimulation of the splanchnic nerve and were studied further.

Each of the 37 medullary neurons studied were examined with respect to their relationship to the cardiac rhythm. We observed a strong relationship (average coherence, 0.81±0.03) between neuronal activity and arterial blood pressure at a cardiovascular frequency of 3.2±0.2 Hz, which represented an average heart rate of 192 beats/min in the 37 neurons studied. An individual example of an rVLM neuron with a coherence of 0.87 at 3 Hz is provided in Fig. 3A. Arterial pulse-triggered averaging likewise showed a strong relationship between the discharge rate of neurons studied in the rVLM and arterial blood pressure (Fig. 3B).

We also examined their responses to altered baroreceptor input using phenylephrine or nitroglycerin, depending on the baseline activity. Nitroglycerin increased the discharge activity of nineteen neurons from 3.7±0.8 to 7.2±1.8 imp/s, while phenylephrine decreased activity consistently in each of the five neurons from 4.6±1.4 to 3.0±1.4 imp/s, indicating that the cells were barosensitive. An original response of an rVLM neuron during treatment with nitroglycerin is displayed in Fig. 3C.

Finally we evoked the responses of twenty-three of the 37 neurons to antidromic stimulation of the IML of the spinal cord to determine if they functioned as premotor sympathetic neurons. We were able to antidromically stimulate each of the 23 neurons studied, thus demonstrating that they were premotor sympathetic in function. The axonal conduction velocity was 7.1±0.6 m/s. Fig. 3D provides an example of collision used to define the bulbospinal nature of these rVLM neurons. We noted that the antidromically-induced action potential collided with the spike evoked by the stimulation of the splanchnic nerve when the interval between the evoked potential and the triggered antidromic potential was reduced by 7.6 ms.
Baseline activity of the rVLM neurons was 3.7±0.7 imp/s. Each of the 37 neurons included in the analysis received input from both the splanchnic nerve and acupoints P5-P6. All three of the neurons tested responded to application of bradykinin (10 µg/ml) to the gallbladder.

Thirty seconds stimulation of acupoints P5-P6 and LI10-LI11 yielded higher levels of rVLM response than LI6-LI7 or K1-B67 (Fig. 4). Similarly, the rVLM neurons responded more to direct stimulation of the median (40±7 imp/30 stim) or deep radial nerves (52±12 imp/30 stim) than to stimulation of the superficial radial nerves (12±6 imp/30 stim, P <0.05). Stimulation at the P5-P6 acupoints and the underlying median nerves caused similar responses (42±11 vs. 40±7 imp/30 stim, respectively) in the six rVLM cells examined. We also observed similar responses in each of eight rVLM neurons studied during percutaneous stimulation at LI6-LI7 acupoints (15±5 imp/30 stim) and direct stimulation of superficial radial nerves (12±6 imp/30 stim). Likewise, responses evoked by stimulation at acupoints LI10-LI11 (56±18 imp/30 stim) and direct stimulation of the deep radial nerves (52±12 imp/30 stim) were not different in five neurons. Stimulation at LI6-LI7 or K1-B67 both produced responses in only 66% of the cells studied.

The relationship between the magnitude of modulatory effect of EA on the visceral pressor response and input on the rVLM neurons was evaluated by regression analysis, comparing responses during stimulation of each of the six acupoints. This analysis demonstrated a significant linear relationship (r² = 0.71) between the change in MAP and evoked responses in the rVLM (Fig. 5).

To determine the influence of prolonged inhibitory effect of EA on the discharge rate in the rVLM, we stimulated acupoints P5-P6, LI4-L7 or LI6-LI7 for 30 min. Electroacupuncture at acupoints P5-P6 and LI4-L7 decreased the splanchnic nerve-evoked neuronal firing rate. Thus, activity was attenuated by 41% during and after EA at P5-P6 and by 12% at LI4-L7. The inhibitory effects of EA at P5-P6 lasted longer than the two other acupoints. On the other hand, EA at P-W did not significantly inhibit the SN evoked rVLM activity. Thus, the extent of EA-inhibition on the neuronal activity paralleled the convergent input observed during brief stimulation of the deep and superficial somatic nerves (Fig. 6).

**Anatomical location of recording and stimulating sites**

All recording sites were confined to an area 1.2-4.5 mm rostral to the obex, 2.5-3.7 mm to the right or left of midline, 0.2-1.4 mm from the ventral surface, lateral to the nucleus inferior olive and pyramidal tracts, as well as ventral and medial to the facial and retrofacial nuclei (Fig. 7). These recording sites are located within the area of the rVLM as described by several authors (6,7,10,11,20,24,38). Five neurons not responsive to convergent inputs of splanchnic and median nerves are indicated with open circles. 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 that is similar to a previous report by Morrison et al, 1985 (44).
DISCUSSION

The term point specificity is used in the clinical practice of acupuncture to identify acupoints that have specific physiological or clinical effects. However, this concept here-to-fore has not been examined rigorously. Therefore, the present study was designed to investigate the influence of electrical stimulation of a number of acupoints located over somatic nerves on the visceral-induced pressor response to address the issue of point specificity and to understand, in part, the underlying medullary mechanism. Furthermore, an important additional goal of this study was to determine if premotor sympatoexcitatory neurons in the rVLM serve as a central site for integration of point specific cardiovascular responses to EA.

The present study demonstrated clear evidence for differential or point specific cardiovascular responses to EA. Furthermore, sympathetic cardiovascular neurons in the rVLM, which receive convergent input from visceral organs, such as the gallbladder, and selected somatic nerves known to be activated during stimulation of acupoints by EA, demonstrated a similar differential evoked response during EA. In line with this observation, EA stimulation of these pathways led to concordant inhibition of medullary neuronal evoked responses. These data show that point specificity exists and that the rVLM serves as one site that potentially can regulate the influence of EA on sympathetic outflow to the cardiovascular system.

We observed that 30 min stimulation of low frequency, low current EA of the deep or superficial (i.e., cutaneous) somatic nerves respectively exerted either a prolonged or no attenuation of the reflex sympatoexcitatory cardiovascular responses. Like the inhibitory influence of EA on the pressor reflexes, we observed that stimulation of acupoints overlying deep compared to superficial somatic nerves evoked significantly more activity in sympathetic cardiovascular neurons of the rVLM. The rVLM neurons receiving differential levels of input from various somatic nerves thus are positioned to integrate somatic and visceral afferent input, to orchestrate rVLM discharge activity during EA, which, in turn, modifies cardiovascular excitatory reflexes.

The rVLM contains many barosensitive reticulospinal neurons that project to the thoracic IML and receives sensory inputs from somatic sensory and visceral afferents (6,25) and serves an important role in somatosympathetic responses (17). This medullary region is located caudal and ventromedial to the facial nucleus, medial and inferior to the retrofacial nucleus and includes the medial and caudal parts of nucleus paragigantocellularis lateralis (41). The rVLM in the cat is limited medially by the gigantocellular field, the inferior olive and the nucleus interfascicularis hypoglossi; is located ventral to the retrofacial nucleus and extends ventrally to the ventral medullary surface (15,23,25,39). Neurons
identified in this study, characterized as sympathoexcitatory and/or cardiovascular in function were located in this region as defined by these general landmarks. The specific relationship of these neurons to the rVLM was confirmed by five other neurons that were found to be not responsive to stimulation of both the splanchnic and median nerves and which were found to be located outside the rVLM.

Three mechanisms potentially may contribute to point specificity. Firstly, neural pathways underlying acupoints appear to account to a large extent for differences in the influence of EA on cardiovascular reflex responses. Our previous work has shown that direct stimulation of the median nerves with low currents and low frequencies causes comparable cardiovascular inhibitory effects as EA at the P5-P6 acupoints (12,32). Others have shown that stimulation of another deep somatic pathway, the deep peroneal nerve, reduces arrhythmias induced by hypothalamic stimulation (21,61). Similarly, the current and previous studies have demonstrated that stimulation of the Zusanli acupoint overlying the deep peroneal nerve reduces the visceral pressor reflex response (35). Conversely, since EA at acupoints LI6-LI7 did not modulate the visceral-cardiovascular reflex, we directly stimulated the superficial radial nerves. Like EA at this acupoint, direct cutaneous nerve stimulation failed to inhibit the reflex response, indicating that we could not explain the absence of a response by technical problems associated with insertion or placement of the acupuncture needle. The differential superficial and deep somatic neural influence of EA on cardiovascular reflex response was confirmed by the absence of an EA effect in response to stimulation of K1-B67, overlying the superficial branches of the terminal tibial nerve on the lower extremity. EA at acupoints LI4-L7 overlying pathways that innervate a combination of both superficial radial and deeper branches of the median nerves demonstrate significant but relatively shorter lasting EA-related cardiovascular influence than stimulation of acupoints such as P5-P6. Deeper nerves such as the median and deep radial nerves provide more input to rVLM neurons than superficial radial and tibial cutaneous nerves. These differences in effect may be related to the number of myelinated and non-myelinated afferents in the peripheral pathways (32). In this latter respect, we have shown that both myelinated and unmyelinated fibers may contribute to the EA response, although twice as many myelinated as unmyelinated somatic afferents are stimulated during low current, low frequency EA at P5-P6 (32). Further studies will be needed to determine the relative importance of Group III vs. Group IV afferents during EA. Thus, the extent of influence of EA at acupoints that potentially may be used to treat cardiovascular diseases depends, at least in part, on the underlying neuronal pathways.

Secondly, there may be a spinal component to the point specific influence of EA. Sympathetic reflexes may display a segmental representation with respect to the spinal level of input. For example, segmental spinal reflex discharge recorded in sympathetic nerves can be elicited by stimulation of specific somatic afferent pathways (8,9). Acupuncture, leading to stimulation of a spinal segment, can inhibit the somatosympathetic reflexes related outflow at the same or a nearby segment, possibly through
an influence of the enkephalin system (36,62). Thus, stimulation of somatic afferent pathways may lead to input at spinal segments which are at or are nearby T1-T6 through which cardiac sympathetic efferents exit (29,54). In this respect, we found that EA at the P5-P6 acupoints, which activate afferents projecting to spinal segments C6-T1 (45) resulted in a stronger EA effect than EA at acupoints S36-S37 overlying afferents that project to segments L4-S3 (1).

Lastly, responses of different regions of the brain that participate in the autonomic reflexes to various somatic afferent inputs, and the effect of this input on various neuromodulators and/or neurotransmitters such as opioids, glutamate, etc. may contribute to the distinct modulatory effect by EA (44). The present study shows clear differences in evoked activity of rVLM neurons that participate in cardiovascular regulation through their influence on sympathetic outflow. We found a strong linear relationship between the cardiovascular influence of EA and the extent to convergent excitation in the rVLM during stimulation of the somatic nerves as well as the overlying acupoints. We have shown previously that rVLM neurons that are excited initially during brief stimulation of somatic afferents display a smaller response to visceral reflex excitation following prolonged somatic nerve stimulation (i.e., EA). We conclude that the extent of excitation of rVLM neurons during brief somatic nerve stimulation correlates to the level of prolonged cellular inhibition during prolonged EA-related stimulation. We speculate that the initial excitatory response may be mediated by a glutamate mechanism, while the later EA-related inhibitory response is mediated by an opioid mechanism (57). We also have shown that opioid receptors in this region, particularly µ- and δ-opioid receptors that respond to β-endorphin, endomorphin and enkephalin (but not dynorphin) are involved intimately in inhibition of cardiovascular function by EA (33). In addition to the rVLM, which represents one important site of central integration during EA’s influence on reflex excitatory responses, other regions such as the arcuate nucleus, periaqueductal gray and nucleus raphe obscurus, in the hypothalamus, midbrain and medulla, respectively, also have been suggested to play a role in the influence of EA on the cardiovascular system (12,28,31,33-35,40,58,60). Future work will need to examine the differential responses and specific neurotransmitter mechanisms relative to point specificity in each of these areas.

Other studies report beneficial effects of EA on cardiovascular-related clinical conditions (14). However, the most effective acupoints and underlying somatic nerves for treating diseases such as hypertension, arrhythmias and myocardial ischemia have not been identified clearly. The present study is the first to demonstrate a difference in the magnitude of influence and duration of cardiovascular response to stimulation of a number of acupoints on the upper and lower extremities that are thought to be useful clinically. Our previous studies have shown long lasting effects of EA at P5-P6 and S36-S37 and have suggested that the influence of the former outlasts the latter acupoints during cardiovascular stimulation in response to gastric distension (12,33,35). The present study suggests that, compared to
acupoints overlying superficial somatic nerves, stimulation of acupoints overlying deep upper or lower extremity somatic nerves during EA can modulate reflex-induced vascular responses to a greater extent and for a longer length of time compared to stimulation of acupoints overlying more superficial neural pathways. These data may have direct clinical implications for the practice of EA.

Certain clinically relevant acupuncture reports point to acupoint specific responses. For example, stimulation of either acupoints P6, S36 or the median or deep peroneal nerves located beneath these acupoints (but not G39 or L7 nor the underlying superficial peroneal or superficial radial nerves) reduces the number of ventricular extrasystoles induced by stimulation of the hypothalamic defense area (21). Although point specificity is an important aspect in the clinical practice of acupuncture, there are many inconsistencies in the usage of specific acupoints. For instance, patients with the same disease frequently receive acupuncture at different acupoints (3). Acupuncturists tend to use acupoints based on his or her own clinical experience, relying on empirical or anecdotal observations reported in older texts and/or the theory of meridians taught by Traditional Chinese Medicine (3,18,42). The present study provides definitive evidence to support the concept of point specific responses during EA, at least with respect to the influence of EA upon cardiovascular excitatory reflexes, and suggests one mechanism, convergent input into rVLM cells that ultimately influence sympathetic outflow; thus, appear to be closely related to the influence of EA on the cardiovascular system.

One potential limitation of the present study is that we studied only a subset (25) of the 37 “cardiovascular” rVLM neurons with respect to antidromic stimulation from the IML. In this regard, we have shown previously that the majority (86%) of cells in the rVLM that receive convergent input from the splanchnic and median nerve can be antidromically driven from the IML (57), suggesting that the majority of rVLM neurons receiving visceral and somatic input function as premotor sympathetic cells. Thus, we predict that many of the other 14 cardiovascular rVLM neurons, which were not examined for antidromic stimulation from the spinal cord IML, may function as premotor neurons.

In conclusion, the present study demonstrates a range of cardiovascular responses to EA that result from stimulating different acupoints, thereby documenting point specific responses. The levels of EA-related modulation of visceral reflex pressor responses are influenced by the anatomical location of somatic nerves beneath the acupoints, with deep nerves exerting strong influence and superficial cutaneous nerves demonstrating little or no attenuation of cardiovascular reflex responses. Brief stimulation (30 s) at acupoints or their underlying neural pathways as well as prolonged stimulation (30 min, EA) evoked a graded rVLM neuronal evoked activity and inhibition of sympathoexcitatory activity. This study thus demonstrates the importance of specific cardiovascular reflex responses to selective acupuncture point stimulation during EA, and suggests that differential input into the rVLM can,
depending upon the acupoints stimulated, inhibit cardiovascular and sympathoexcitatory medullary neuronal activity as one mechanism in the CNS that underlies this effect.

ACKNOWLEDGMENT

This work was supported by the National Institute of Health, Bethesda, MD, grants HL-36527, HL-72125 and HL-63313 and the Larry K. Dodge Endowed Chair (JCL). The authors gratefully acknowledge Teadora Agoncillo and Vu Thai Nguyen for their technical assistance, Dr. Zhongyuan Shen, Shanghai University of Traditional Medicine and Mr. Yingxian Cao, Department of Physiology, Shanghai Medical College, Fu Dan University, China for the development of software.
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Figure 1. Histograms displaying increases in mean arterial blood pressure (MAP) during repeated stimulation of visceral afferents on the gallbladder with bradykinin applied every 15 min. Panel A shows consistent increases in MAP in the absence of EA. Panels B and C demonstrate prolonged (75 and 45 min, respectively) reduction of the pressor response after 30 min of EA at acupoints P5-P6 (overlying the median nerve) and LI10-LI11 (overlying the deep radial nerve). Panels D and E demonstrate shorter lasting decreases of the pressor reflex evoked by stimulation of the visceral afferents during and after 30 min EA at the LI4-L7 (overlying branch of median nerve and superficial radial nerve) and S36-S37 (overlying peroneal nerve) acupoints. Thirty min of EA at LI6-LI7 (overlying superficial radial nerve) and K1-B67 (terminal branches of the tibial nerve) acupoints did not attenuate the pressor response (Panels F and H). Similarly, direct stimulation of the superficial radial nerve for 30 min did not modulate the pressor reflex (Panel G). Asterisk indicates decreased pressor responses compared with the two responses before onset of EA. Numbers below bars indicate baseline blood pressure before stimulation of the gallbladder.

Figure 2. Efficacy and duration of the inhibitory EA effect are displayed in panels A and B. P5-P6 (J-N) and LI10-LI11 (Sh-Q) demonstrated the largest reduction in percent change of the increase in MAP (Panel A). The pressor responses of EA at P5-P6 and LI10-LI11 were significantly (asterisks) inhibited, i.e., smallest change of the increase in MAP (shown in Fig. 1) compared with LI6-LI7 (P-W), SRN, and K1-B67 (Y-Z). Panel B demonstrates duration of the EA modulation among the sets of acupoints. Effects of EA at acupoints P5-P6 and LI10-LI11 were longer lasting than LI4-L7 (H-L) and S36-S37 (Z-S), as noted with asterisk. SRN, P-W and Y-Z indicate superficial radial nerve and acupoints LI6-LI7 and K1-B67, respectively.
Figure 3. Methods of characterization of rVLM neurons including frequency and time domain analyses of relationship between the discharge of rVLM neuron and arterial blood pressure (BP). Autospectra (AS) of BP and rVLM neuronal activity and corresponding coherence function of 0.87 are provided in Panel A and arterial pulse-triggered analysis of rVLM activity (average based on 100 trials, bin widths of 12 ms) is shown in Panel B. Measurement of baroreceptor input showing a change in blood pressure and spontaneous discharge of an rVLM neuron after administration of nitroglycerin shown in Panel C. This neuron also responded to stimulation of the splanchnic and superficial radial nerves and acupoints P5-P6, LI4-L7, LI6-LI7 and K1-B67. Panel D displays collision during antidromic stimulation of the IML at T3 of the spinal cord. The distance from the IML at T2 to this rVLM neuron was 40 mm, the onset latency was 4.6 ms and the calculated conduction velocity was 8.7 m/s. The refractory period of this neuron was 2 ms resulting in a critical interval of 11.2 ms. In first sweep, the neuron was activated by stimulation of the splanchnic nerve and the IML. The second sweep shows collision as we reduced the interval between orthodromic (splanchnic nerve-induced activity) and antidromic (IML-generated discharge) impulses. This sweep displays only one spike (seen on the left of the artifact induced by antidromic stimulation of the IML). The spike that should have occurred with IML stimulation was cancelled by the discharge evoked by splanchnic nerve stimulation. In third sweep, the antidromic spike reappeared as the interval was increased. ~ Indicates time of stimulation of the IML. ↓ Indicates time of stimulation of the splanchnic nerve.

Figure 4. Neurograms of evoked activity during stimulation at 2 Hz of the splanchnic nerve (SN), P5-P6, LI4-L7 and LI6-LI7 showing different degrees of convergent input. Tracings above the neurograms show stimulation artifacts (above) in relation to the neurogram (below) during extracellular recording (Panel A). Bar histograms (Panel B) show range of convergent input into rVLM neurons. Heights of bars represent average evoked activities during 30 stimuli of SN, median nerve or one of the six sets of acupoints, determined from responses of cardiovascular neurons in rVLM. EA at acupoints P5-P6 and
LI10-LI11 induced the highest level of input and hence provided the greatest opportunity to modulate sympathoexcitatory reflexes at level of the rVLM. Numbers inside bars indicate the number of responsive cells relative to numbers of cells studied. Asterisks indicate significantly different (P<0.05) input compared with P5-P6 acupoints. † Indicates significantly different (P<0.05) input compared with LI10-LI11.

Figure 5. Regression analysis demonstrating a correlation (r² = 0.71, P < 0.05) between change in MAP and convergent input on sympathetic rVLM neurons. We noted a linear association between the level of convergent input in cardiovascular-related rVLM neurons and the inhibitory effects of EA (change in MAP) on sympathoexcitatory pressor reflexes.

Figure 6. Prolonged inhibition of cardiovascular medullary neurons during and after electroacupuncture (EA) at P5-P6 (J-N), LI4-L7 (H-L) and LI6-LI7 (P-W) displayed as the percentage change. Bar histograms indicate significant change (∼) lasting 70 min by EA at acupoints P5-P6. RVLM activity described as the % change reports the increase in activity in response to splanchnic nerve stimulation for each of the four groups, relative to baseline or to pre EA stimulation period for each group. The degree of inhibition of neuronal activity compared to repeated evoked activity in the absence of EA (control group) is greater at P5-P6 than LI4-L7.

Figure 7. Composite map is displaying the sites of neuronal recordings (∼) in and (ο) outside rVLM. Sites are recordings of 37 neurons during examination for convergence of splanchnic nerves and somatic input from stimulation at different acupoints. Sections represent combinations of medullary planes rostral to obex. Inferior olivary nucleus (ION), alaminar spinal trigeminal nucleus (5SP) and retrofacial nucleus (RFN) are shown for reference.
<table>
<thead>
<tr>
<th>Acupoint*</th>
<th>Neural Pathway</th>
<th>Anatomical Location</th>
</tr>
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<tbody>
<tr>
<td>P5-P6</td>
<td>Jiangshi-Neiquan (J-N)</td>
<td>Median nerve proximal to accessory carpel pad of forelimb between ligaments of flexor carpi radialis and palmaris longus</td>
</tr>
<tr>
<td>LI 10-LI11</td>
<td>Shousanli-Quchi (Sh-Q)</td>
<td>Deep radial nerve distal to and on elbow between extensor carpi radialis longus and brevis</td>
</tr>
<tr>
<td>LI4-L7</td>
<td>Hegu-Lique (H-L)</td>
<td>Branch of median and Superficial radial nerve Hgu - dorsal aspect of paw, between midpoints of first and second metacarpal bones and between first dorsal interosseous muscle and adductor pollicis of thumb. Lique - radial side of forelimb proximal to accessory carpel pad between abductor pollicus longus and tendon of brachio-radialis.</td>
</tr>
<tr>
<td>S36-S37</td>
<td>Zusanli-Shangjuxu (Z-S)</td>
<td>Deep peroneal nerve anterolateral side of hindlimb near anterior crest of tibia below knee underneath tibialis anterior muscle.</td>
</tr>
<tr>
<td>LI6-LI7</td>
<td>Pianli-Wenlui (P-W)</td>
<td>Superficial radial nerve radial side of dorsal surface on lower one third of forelimb between abductor pollicus longus, extensor pollicis brevis and longus.</td>
</tr>
<tr>
<td>K1-B67</td>
<td>Youngquan-Zhiyin (Y-Z)</td>
<td>Terminal branch of the tibial nerve Youngquan - center of metatarsal pad of hindlimb. Zhiyin - lateral side digit 5 above claw.</td>
</tr>
</tbody>
</table>

* refers to World Health Organization nomenclature and Pinyin (Chinese phonetic alphabet) names for acupoints.
Fig. 1
Fig. 2

A. % Change of \( \Delta MAP \)

- \( \Delta MAP \) compared with P5-P6 and LI10-LI11

B. Duration of EA effect (min)

- \( \Delta MAP \) compared with P5-P6 and LI10-LI11

- Control
- P5-P6
- LI10-LI11
- LI4-L7
- S36-S37
- LI6-LI7
- SRN
- K1-B67

\( n=7 \) \( n=6 \) \( n=7 \) \( n=8 \) \( n=8 \) \( n=6 \) \( n=5 \) \( n=5 \)
Fig. 3
Fig. 4
Fig. 5

MAP change (mmHg)

RVLM evoked activity (imp/30 stim)

P5-P6  LI10-LI11  S36-S37  LI4-L7  LI6-LI7  K1-B67
Fig. 6