Afferent mechanisms underlying stimulation modality-related modulation of acupuncture-related cardiovascular responses

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employed during its application. However, because there remain many unclear issues concerning the influence of acupuncture stimulation modality, this study was aimed at further evaluation of input variables to establish a better understanding of the parameters that regulate the response to acupuncture. We tested the following hypotheses: 1) EA is more effective than MA because it stimulates somatic afferents to a greater degree than MA in modulatory cardiovascular reflex responses and 2) there is greater inhibition of sympathoexcitatory pressor reflexes with EA using low-frequency stimulation, acupoints overlying deep somatic nerves, or simultaneous stimulation of two sets of effective acupoints. A preliminary report of this work has been published (42).

METHODS

Surgical Procedures

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of the University of California, Irvine, CA. The study conformed to the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings. Studies were performed on adult Sprague-Dawley male rats (400–600 g). After an overnight fast (18 h), anesthesia was induced with ketamine (100 mg/kg im) and was maintained with α-chloralose (50–60 mg/kg iv). Additional doses of α-chloralose (25–30 mg/kg iv) were given as necessary to maintain an adequate depth of anesthesia. The right jugular vein was cannulated for administration of sodium bicarbonate and α-chloralose. The trachea was intubated, and respiration was monitored with a ventilator (model 661, Harvard Apparatus). The right or left carotid artery was cannulated and attached to a pressure transducer (Statham P23 ID, Gould) to monitor systemic BP. HR was derived from the pulsatile BP signal. Arterial blood gases and pH were measured periodically with a blood-gas analyzer (model ABL5, Radiometer America) and were kept within normal physiological limits (PCO\textsubscript{2} 30–40 Torr and PO\textsubscript{2} >100 Torr) by adjusting the ventilatory rate or volume and enriching the inspired O\textsubscript{2} supply. Arterial pH was maintained between 7.35 and 7.43 by infusion of a solution of 8% sodium bicarbonate. Body temperature was kept between 36 and 38°C with a heating pad and lamp.

A 3-cm (unstressed dimension) latex balloon was attached to a polyurethane tube (3-mm diameter) that was inserted into the stomach through the mouth and esophagus. A syringe was attached to the cannula to inflate and deflate the balloon with air. Distension pressures were selected to fall within the range that a rat normally experiences during ingestion of food and fluids in a single meal (2, 3, 10, 11). Within 5–10 s of inflation, we noted an increase in systemic arterial BP. In the single instance when the balloon was not in the stomach but remained in the esophagus, the pressor response was much larger; we eliminated this animal from our data.

Experimental Procedure

After the surgical procedure, we allowed a 30-min stabilization period before beginning the experimental protocols. The balloon was inflated every 10 min throughout each experiment by injecting 5–10 ml of air for 30 s, a volume that induced a distension pressure of 10–20 mmHg (3, 11). The volume of air used for distension was maintained constant for each animal throughout the protocol. Ten-minute intervals between inflations prevented tachyphylaxis of the cardiovascular responses. After the maximal cardiovascular pressor response was observed, air was withdrawn from the balloon. After the completion of each experiment, rats were euthanized with intravenous KCl under deep anesthesia; the stomach then was exposed to verify the location of the balloon. Only animals in which the balloon was observed to be within the stomach were used for data analysis. BP and HR responses were recorded and were analyzed offline with a Pentium III computer and data-acquisition software Power Lab (ADInstruments).

Somatic Afferent Recordings

Single-unit activity of somatic afferent fibers was recorded as described previously (15, 24). In brief, the median nerve of rat was isolated in the upper forelimb near the humerus and covered with warm mineral oil, desheathed, and split into fine nerve filaments under a surgical microscope (model OPMI 1-FC, Zeiss). The peripheral end of a filament was draped over one pole of a bipolar recording electrode attached to a high-impedance probe. The other pole of the electrode was grounded with a saline-saturated cotton thread to the surrounding tissue. Discharge activity of each afferent fiber was amplified (model P511 preamplifier, Grass), then processed through an audio amplifier (model AM8B, Grass Instruments), and displayed on a storage oscilloscope (model 2201, Tektronix) to allow discrimination. The discharge frequency of afferents was analyzed using data-acquisition and analysis software (Spike version 4.18, Cambridge Electronic Design), and a histogram was created for each afferent.

To ensure that the signals measured were from afferent fibers, the nerve action potential was evoked by mechanical manipulation of receptive fields in the paw. Conduction distance was measured with a thread placed from the stimulating to the recording electrode along the course of the median nerve. Conduction time was determined by measuring the latency from the signal of electrical stimulation (ES) to the corresponding action potential in the afferent. Conduction velocity of the afferent was calculated by dividing the conduction distance by conduction time. Classification followed established categories from previous studies on rats (16, 20, 21). Fibers with conduction velocities <2 m/s were classified as C fibers, whereas those with velocities between 20 and 22 m/s were considered to be A\textsubscript{δ}-fibers.

Experimental Protocols

Protocol 1: Control responses. TIME CONTROL. Six rats were subjected to 11 repeated periods of gastric distension without acupuncture; each distension lasted 30 s and was repeated at 10-min intervals over 110 min while reflex BP responses and HR were monitored. Acupuncture needles were not inserted, because it has been suggested that even simple insertion of such needles may cause an acupuncture-like effect (36).

SHAM ACUPUNCTURE. To evaluate the possibility that simple insertion of needles can cause an acupuncture response, in seven rats, after the recording of two reproducible control responses to gastric distension, 32-gauge stainless steel acupuncture needles (Suzhou Medical Appliance) were inserted into Jianshi-Neiguan (P 5–6) acupoints overlying the median nerve. Needles were inserted perpendicularly to a depth of 3–5 mm. The correct positioning of acupuncture needles in human subjects relies on their feeling of “heaviness” associated with ES of the needles when properly positioned at the acupoint (7). However, this information is not available in animals. Therefore, our criterion for correct needle positioning relied on our observation of a slight repetitive flexion of the paw during ES, as in our laboratory’s previous study (7). The period of this initial ES to ensure proper needle placement lasted no more than 30 s. Once we confirmed the proper location, we left the needles at P 5–6 without electrical stimulation or manual manipulation for 30 min.

CONTROL ACUPUNCTS. Like others (31), we believe that the best control for an acupoint is to stimulate another acupoint on another meridian that has been reported to have a different function. This has been termed the strong control (31). In the present study, we chose Pianli-Wenliu (LI 6–7) as control acupoints because they are near P 5–6 yet are located along another meridian (large intestine). After the recording of two reproducible control responses to gastric distension in six rats, acupuncture needles were inserted bilaterally into LI 6–7 acupoints, overlying the superficial radial nerves. These needles were

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stimulated electrically using low current and low frequency (0.3–0.5 mA, 0.5-ms duration, 2 Hz) for 30 min by using an electrical stimulator with a stimulus isolation unit (model 88, Grass).

Protocol 2: MA and EA at P 5–6. Reflex. After the establishment of two consistent BP responses to gastric distension in eight rats, needles were inserted bilaterally into P 5–6 acupoints and were twisted manually at a frequency of ~2 Hz for 2 min. We repeated the 2-Hz, 2 min of needle stimulation every 10 min for 30 min, while gastric distension was repeated every 10 min for 110 min. Alternatively, needles were inserted bilaterally at P 5–6 and were stimulated...
electrically at 2 Hz, 0.3–0.5 mA, 0.5-ms duration, and for 2 min in seven rats. The same protocol for gastric distension as noted for MA stimulation was used to evaluate the cardiovascular responses.

SOMATIC AFFERENTS. To assess the responses of Aδ- and C fibers to ES and manual stimulation (MS) using the same stimulation previously noted above, we recorded action potentials of 79 somatic afferents in the median nerve during 60-s periods of MS or ES in two rats.

Protocol 3: EA stimulation frequency. REFLEX. Frequency responses to P 5–6 EA (0.3–0.5 mA, 0.5 ms) at 2 Hz (n = 7), 40 Hz (n = 6), and 100 Hz (n = 6) were evaluated. In this protocol, during EA and for 60 min after its termination, responses to gastric distension were recorded every 10 min. The 60-min period after EA was used to assess recovery of the inhibitory effects of EA on the cardiovascular response.

SOMATIC AFFERENTS. We recorded action potentials of 79 somatic afferents from the median nerve during 60 s of ES (0.3–0.5 mA, 0.5 ms) at 2, 20, 40, and 100 Hz in two rats.

Protocol 4: Acupoint location and combination. Three groups of animals were employed to evaluate the influence of EA on the gastric distension cardiovascular reflex response. Acupoint specificity was examined by evaluating the responses to S 36–37 overlying (stomach meridian, overlying deep peroneal nerve, 2 Hz, n = 6) or H 6–7 (heart meridian, overlying ulnar nerve, 2 Hz, n = 5). Acupoint combination was evaluated by observing the influence of simultaneous stimulation of P 5–6 and S 36–37 (2 Hz, n = 8).

**Statistical Analysis**

Means and SEs of the mean arterial pressure and HR at rest were compared over time using a repeated-measures ANOVA followed by Tukey’s test. Gastric distension responses also were analyzed with one-way repeated-measures ANOVA followed post hoc by Tukey’s test to compare BP responses before, during, and after MA and EA in each group. Responses of somatic afferents during ES and MS were compared using the Student’s t-test. The number and discharge activity of afferents during EA at 2, 10, and 20 Hz were compared with the χ² test and a one-way ANOVA. Statistical calculations were performed with SigmaStat software (Jandel Scientific Software, San Rafael, CA). The 0.05 probability level was chosen to determine statistical significance.

**RESULTS**

**Protocol 1: Control Responses**

The BP response to gastric distension in the time control group was consistent over the 110-min period of evaluation in the absence of acupuncture stimulation (Fig. 1A, n = 5). Additionally, neither acupuncture needle insertion at P 5–6

![Fig. 3. Representative tracings of somatic C-fiber afferent in median nerve (conduction velocity = 0.3 m/s). Arrow, stimulus artifact. Action potential of afferent (●) activated by 2-Hz electrical stimulation (ES) in A is shown in a. B: neural activity of afferent during manual stimulation (MS) at 2 Hz.](image)

**Protocol 3: EA stimulation frequency.** REFLEX. Frequency responses to P 5–6 EA (0.3–0.5 mA, 0.5 ms) at 2 Hz (n = 7), 40 Hz (n = 6), and 100 Hz (n = 6) were evaluated. In this protocol, during EA and for 60 min after its termination, responses to gastric distension were recorded every 10 min. The 60-min period after EA was used to assess recovery of the inhibitory effects of EA on the cardiovascular response.

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**Fig. 4.** Responses of median nerve somatic afferents to ES and MS. Histograms show impulse (imp) activity of somatic afferents to 10 s of 2-Hz ES (total number action potentials: 50; A) or 2-Hz MS (total action potentials: 54; B). Expanded tracing to right displays spike activity after electrical artifacts. C: group data of all Aδ- and C fibers in two rats responding to ES (n = 79 afferents) and MS (n = 64 afferents). Values are means ± SE; n, no. of rats. *P < 0.05: ES and MS vs. basal, prestimulation.
without stimulation for 30 min (Fig. 1B) nor 30 min EA at an inactive acupoint (LI 6–7) (Fig. 1C) influenced the gastric distention-induced pressor responses over a similar time period. HR in all of the time control groups was unchanged by gastric distension.

Protocol 2: EA and MA at P 5–6: Reflex and Somatic Afferent Responses

When EA (A) and MA at P 5–6 over the median nerve on the forehands were matched for frequency and duration of application, we observed an immediate and a prolonged reduction of the cardiovascular BP response to gastric distension lasting 50–60 min (Fig. 2). The extent of inhibitory effects of the two modalities were similar (MA: 33% vs. EA: 36%), but the inhibitory influence of EA was marginally (10 min) longer than MA. The activity of 132 afferent fibers was recorded during mechanical stimulation of receptive fields on the paws of two rats. ES at 0.3–0.5 mA and 2 Hz activated 79 fibers of the 132 afferents, including (18 Aδ-fibers, 61 C fibers) (Fig. 3, A and B, and Fig. 4, A and B). MS at ~2 Hz activated 64 fibers of the 79 afferents (14 Aδ-fibers, 50 C fibers) that responded to ES. We observed a very low spontaneous discharge activity of these afferents (Fig. 4, A, and B). We found no significant difference in discharge activity of the afferents during ES or MS (Fig. 4C, 6.0 ± 1.4 vs. 5.9 ± 1.1 impulses/s).

Protocol 3: EA Stimulation Frequency: Reflex and Somatic Afferent Responses

Thirty minutes of P 5–6 EA at 2 Hz significantly inhibited the gastric-cardiovascular pressor reflex (Fig. 5A), whereas a similar period of EA at 40 (Fig. 5B) or 100 Hz (Fig. 5C) did not alter the response. We observed significantly larger somatic afferent responses during 2-Hz (Fig. 6A) vs.10-Hz ES (Fig. 6B). There was a reciprocal relationship between the frequency of stimulation and the average responsive afferent fibers per stimulus (Fig. 6C). In total, afferent stimulation at 2, 10, and 20 Hz activated 79, 29, and 13 fibers, respectively. We were unable to record the somatic afferent action potentials during 40- and 100-Hz EA stimulation because of interference from the stimulation artifact.

Protocol 4: Acupoint Location and Combination

EA at H 6–7 (Fig. 7A) and EA at S 36–37 (Fig. 7C) similarly inhibited the visceral reflex (44 and 39%, respectively). However, combined stimulation of two sets of acupoints at P 5–6 and S 36–37 caused no additive or synergistic response compared with EA at P 5–6 or S 36–37 alone (Fig. 7D). Figure 8 summarizes the attenuation of gastric distension-induced pressor response during EA at P 5–6, S 36–37, and with combined stimulation of the two sets of acupoints. Although we predicted that EA at two sets of acupoints would
produce greater inhibitory effect on the pressor response than stimulation at either P 5–6 or S 36–37 alone (−24.3 ± 2.5 vs. −13.9 ± 2.0 or −11.8 ± 1.8 mmHg), respectively, we observed similar responses induced by simultaneous stimulation at P 5–6 or S36–37.

DISCUSSION

There are several novel observations made in the present study. First, neither simple needle insertion at an active acupoint nor stimulation along a meridian at inactive (i.e., noncardiovascular) acupoint (39) modulates sympathoexcitatory cardiovascular response to gastric distension. Thus both forms of stimulation can serve as adequate controls for acupuncture. Second, MA is as effective as EA with respect to the extent of their modulatory influences on the reflex sympathoexcitatory responses. Third, no additive or synergistic influence occurs when two active acupoints are stimulated simultaneously. Fourth, the modulatory cardiovascular effectiveness of low- vs. high-frequency ES and MS stimulation is related to the ability of low- but not high-frequency ES to activate Aβ- and C-fiber somatic afferents.

Most acupuncturists believe that even simple insertion of acupuncture needles at active acupoints can cause acupuncture-like effects (36, 37). However, the present study shows that in the anesthetized rat needle insertion at P 5–6 without any form of stimulation does not influence the pressor response. Our somatic afferent activity data support this observation because insertion of the needle without manipulative stimulation did not evoke action potentials in somatic afferents. Thus this form of sham acupuncture does not influence cardiovascular excitatory reflex responses in anesthetized animals and would be unlikely to exert effects in unanesthetized animals or humans because the anesthesia used in this experiment acts centrally rather than on the peripheral nervous system.

We chose LI 6–7 as control acupoints because they are near P 5–6, yet are located along another meridian. As predicted, EA at LI 6–7 did not inhibit the pressor response. Therefore, either needle insertion without stimulation at active acupoints or acupuncture at control acupoints appears to serve as an adequate control for acupuncture in anesthetized animals. A previous study suggested that sham acupuncture may cause analgesia in 40–50% of subjects compared with 60–75% of subjects undergoing real acupuncture (41). Furthermore, Middlekauff and colleagues (32) reported that sham acupuncture at nonacupoints in patients had a quantifiable effect on the BP response to mental stress. Our data would suggest that these responses in awake human subjects are due to conscious perception evoking placebo responses rather than those related to activation of somatic afferents, the physiological basis for true acupuncture effects (28).

The acupoint H 6–7 overlying the ulnar nerve were selected for study because they are located along the “heart” meridian and are believed to be effective in treating cardiovascular diseases such as hypertension, coronary heart disease, angina pectoris, arrhythmias, and cardiac insufficiency (9). However, little is known about the influence of EA at H 6–7 on the sympathoexcitatory cardiovascular reflex responses such as gastric distension. We observed that both EA at H 6–7 and P 5–6 inhibited the reflex pressor response in a similar fashion. Interestingly, we also found that EA at S 36–37 inhibited the pressor response, although these acupoints are located on an entirely different meridian, the stomach meridian overlying the peroneal nerve in the leg. Thus these data suggest that the point specificity plays an essential role in the regulation of sympathoexcitatory cardiovascular responses. Recently, our laboratory demonstrated that EA stimulation manifests graded rostral ventral lateral medullar (rVLM) responses during stimulation of different acupoints that exhibit site-specific influences on visceral (gallbladder) reflex sympathoexcitatory cardiovascular responses in cats, suggesting that the rVLM plays a role in acupoint-specific inhibition of cardiovascular reflex responses by acupuncture (39).

In clinical practice, a common mode of needle stimulation is manual rotation of the acupuncture needle. MA stimulation can cause both pressor and depressor reflex responses in human subjects (1). Electroacupuncture also can induce changes in pressor reflexes (30). It is not clear whether MA and EA share similar physiological mechanisms. Our data show when the two modalities are matched with respect to low frequency and ent.
duration, MA produces similar inhibitory effects as low-frequency EA with respect to their influences on sympathoexcitatory cardiovascular response to gastric distension. The mechanism of the similar responses was evaluated in our afferent study, which showed that manual and electrical stimulation at P 5–6 similarly activate somatic Aδ- and C-fiber afferents in the median nerve.

A few previous studies examining responses to different frequencies of EA have reached different conclusions. For example, acupuncture analgesia can be induced by either low-frequency stimulation, such as 2 or 4 Hz, or high-frequency stimulation, such as 100 or 200 Hz (17–19). However, studies examining the mechanisms of EA-induced analgesia have shown that the central nervous system responds differently to EA at different frequencies. In this respect, stimulation with 2-Hz EA is thought to mobilize enkephalin, whereas 100 Hz may release dynorphin in both animals and human beings (17–19). Little is known about frequency-dependent EA cardiovascular responses. In the present study, we found that inhibition of the pressor response depends on the frequency of EA (P 5–6, S 36–37; C) compared with EA at Neiguan-Jianshi acupoint (P 5–6, B). D: response to EA at 2 sets of acupoints, P 5–6 and S 36–37, *P < 0.05: during and after EA vs. before EA. Numbers below each bar represent baseline blood pressures (means ± SE); n, no. of rats.

Fig. 7. Gastric distension-related blood pressure responses to EA at different acupoints and to a combination of acupoints and show EA influences at Yinxi-Shenmen (H 6–7; A) acupoints and Zusanli-Shangjuxue (S 36–37; C) compared with EA at Neiguan-Jianshi acupoint (P 5–6, B). D: response to EA at 2 sets of acupoints, P 5–6 and S 36–37. *P < 0.05: during and after EA vs. before EA. Numbers below each bar represent baseline blood pressures (means ± SE); n, no. of rats.

Fig. 8. Inhibitory effect of EA at 1 or at 2 acupoints on pressor responses evoked by gastric distension. Bar graphs indicate the reduction in MAP change. Pred and Obs, predicted and observed blood pressure changes induced by EA at P 5–6 + S 36–37, respectively. Predicated data calculated by simply combining data from EA at P 5–6 and S 36–37. Values are means ± SE.
ES. Stimulation at low (2 Hz), but not mid (40 Hz) and high (100 Hz) frequencies inhibited the gastric cardiovascular reflex. Further evaluation showed that low-frequency (2 Hz) ES activated many more somatic afferents than high-frequency stimulation such as 10 and 20 Hz. We were unable to evaluate the afferent responses to higher frequencies (40 and 100 Hz) due to interference from the signal artifact. The electrical artifact masked the action potentials of somatic afferents evoked by ES because the duration of the stimulation artifact was 25 ms and the interstimuli intervals were 25 and 10 ms, when the frequencies of stimulation were 40 and 100 Hz, respectively. However, it is unlikely that there was significant afferent activation at higher frequencies because there was such a large fall off in response between 2 and 20 Hz. Overall, these data suggest that the extent of activation of somatic afferents during EA at different frequencies contributes substantially to EA-related modulation of excitatory cardiovascular reflex responses induced by distension of the rat’s stomach. Most therapists employ acupuncture by needling at several locations because they believe there is an additive response to stimulating several acupuncture points simultaneously (41). Our results, however, show that stimulation of two sets of acupuncture points that can each exert strong individual inhibitory effects on the reflex pressor responses does not enhance the EA-related response, suggesting that there is no synergism at least under the conditions imposed by the present study. This observation casts into question the rationale for using multiple acupuncture points. However, further investigation of this issue is warranted. In conclusion, low-frequency MA and EA at P 5–6 significantly modulate reflex excitatory cardiovascular responses induced by gastric distension in rats. Low-frequency ES and MS using acupuncture needles similarly activated the Aβ- and C-fiber somatic afferents, indicating that sensory neural input is an important mechanism underlying the acupuncture-related inhibitory cardiovascular regulation. Low- but not mid- or high-frequency EA inhibited the sympathetic cardiovascular reflex, suggesting a frequency-specific influence. The mechanism appears to be related to the extent of activation of somatic afferents since low-frequency ES activated more afferents than mid- and high-frequency stimulation. In addition, we observed that the inhibitory cardiovascular effect of EA was not enhanced by simultaneously stimulating two effective acupuncture points, casting into question the practice by acupuncturists of stimulating multiple points to achieve a greater clinical response. There are many methods of practicing acupuncture, possibly even as many variations in techniques as there are acupuncturists, with subtle or great differences between individual practices. The data in this study provide a greater understanding of several of these different therapeutic techniques used in clinical acupuncture to treat conditions associated with reflex sympathoexcitation and BP elevation.

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