Short-term electroacupuncture at Zusanli resets the arterial baroreflex neural arc toward lower sympathetic nerve activity

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Michikami, Daisaku, Atsunori Kamiya, Toru Kawada, Masashi Inagaki, Toshiaki Shishido, Kenta Yamamoto, Hideto Ariumi, Satoshi Iwase, Junichi Sugenoya, Kenji Sunagawa, and Masaru Sugimachi. Short-term electroacupuncture at Zusanli resets the arterial baroreflex neural arc toward lower sympathetic nerve activity. Am J Physiol Heart Circ Physiol 291: H318–H326, 2006. First published February 24, 2006; doi:10.1152/ajpheart.00975.2005.—Although electroacupuncture reduces sympathetic nerve activity (SNA) and arterial pressure (AP), the effects of electroacupuncture on the arterial baroreflex remain to be systematically analyzed. We investigated the effects of electroacupuncture of Zusanli on the arterial baroreflex using an equilibrium diagram comprised of neural and peripheral arcs. In anesthetized, vagotomized, and aortic-denervated rabbits, we isolated carotid sinuses and changed intra-carotid sinus pressure (CSP) from 40 to 160 mmHg in increments of 20 mmHg/min while recording cardiac SNA and AP. Electroacupuncture of Zusanli was applied with a pulse duration of 5 ms and a frequency of 1 Hz. An electric current 10 times the minimal threshold current required for visible muscle twitches was used and was determined to be 4.8 ± 0.3 mA. Electroacupuncture for 8 min decreased SNA and AP (n = 6). It shifted the neural arc (i.e., CSP-SNA relationship) to lower SNA but did not affect the peripheral arc (i.e., SNA-AP relationship) (n = 8). SNA and AP at the closed-loop operating point, determined by the intersection of the neural and peripheral arcs, decreased from 100 ± 4 to 80 ± 9 arbitrary units and from 108 ± 9 to 99 ± 8 mmHg (each P < 0.005), respectively. Percutaneous denervation eliminated the shift of neural arc by electroacupuncture (n = 6). Decreasing the pulse duration to <2.5 ms eliminated the effects of SNA and AP reduction. In conclusion, short-term electroacupuncture resets the neural arc to lower SNA, which moves the operating point toward lower AP and SNA under baroreflex closed-loop conditions.

Although there are many clinical case reports (21, 30, 32, 39, 40, 42), the effects of electroacupuncture on cardiovascular regulation remain to be systematically investigated. There has been a recent renewal of interest in the inhibitory effects of electroacupuncture of the Zusanli acupoint on the cardiovascular system, including reductions in arterial pressure (AP), heart rate, (3, 15, 16), and sympathetic nerve activity (SNA) (25, 42). Such inhibitory effects are observed during low-frequency (<20 Hz) electroacupuncture. Because the arterial baroreflex is one of the most important control systems that stabilize AP, we quantified the effects of electroacupuncture on the arterial baroreflex over an entire operating range. Systematic analysis would help to assess the possible utility of electroacupuncture as a treatment modality for certain cardiovascular diseases with vagolytic and sympathothetic states (26, 38).

One of the best ways to quantitatively analyze changes in the arterial baroreflex over an entire operating range may be analysis using a baroreflex equilibrium diagram (10, 23, 31) (see Appendix for details). Briefly, the baroreflex equilibrium diagram consists of a neural arc representing SNA as a function of baroreceptor input pressure and a peripheral arc representing AP as a function of SNA. The intersection of the two arcs corresponds to an operating point of the AP regulation under baroreflex closed-loop conditions. Considering the reduced AP and SNA found in previous studies, we hypothesized that short-term electroacupuncture resets the arterial baroreflex neural arc to lower SNA. In the present study, to test this hypothesis, we constructed a baroreflex equilibrium diagram with neural and peripheral arcs in anesthetized rabbits. The present findings indicate that electroacupuncture resets the baroreflex neural arc to lower SNA, moving the closed-loop operating point toward lower AP and SNA.

MATERIALS AND METHODS

Surgical Preparation

Animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Physiological Society of Japan. Twenty-two Japanese White rabbits weighing 2.4–3.3 kg were anesthetized via intravenous injection (2 ml/kg) with a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml) and mechanically ventilated with oxygen-enriched room air. Supplemental doses were injected as necessary (0.5 ml/kg) to maintain an appropriate level of anesthesia. Body temperature was maintained at ~38°C with a heating pad. AP was measured by using a high-fidelity pressure transducer (SPC-330A, Millar Instruments, Houston, TX) inserted via the left femoral artery. To record cardiac SNA, we exposed the left cardiac sympathetic nerve through a midline thoracotomy and attached a pair of stainless steel wire electrodes (Bioflex wire AS633, Cooner Wire, Chatsworth, CA) to the nerve. The nerve fibers peripheral to the

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electrodes were sectioned to eliminate afferent signals from the heart. To insulate and fix the electrodes, the nerves and electrodes were secured with silicone glue (Kwik-Sil, World Precision Instruments, Sarasota, FL). The preamplified nerve signals were band-pass filtered at 150–1,000 Hz, full-wave rectified, and low-pass filtered at a cutoff frequency of 30 Hz by using analog circuit. After that, the neural signals were recorded at a sampling rate of 200 Hz using a 12-bit analog-to-digital converter. Pancuronium bromide (0.1 mg/kg) was administered to prevent contaminating muscular activities. At the end of the experiment, the experimental animals were killed by an overdose of intravenous pentobarbital sodium, and the background noise level of SNA was determined postmortem.

Sixteen of the 22 rabbits were used in protocol 1 (protocols 1-1, 1-2, and 1-3), and the remaining 6 rabbits were used in protocols 2, 3, and 4. In 10 of the 16 rabbits for protocols 1-2 and/or 1-3 described below, we isolated both carotid sinuses from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus regions. The isolated carotid sinuses were filled with warmed physiological saline through catheters inserted via the common carotid arteries. The intracarotid sinus pressure (CSP) was controlled by a servo-controlled piston pump (model ET-126A, Labworks, Costa Mesa, CA). In the baroreflex-open loop experimental settings, bilateral vagal and aortic depressor nerves were sectioned at the neck to minimize reflex effects from cardiopulmonary regions and the aortic arch.

Electroacupuncture

Two stainless steel needles were inserted at the one-fifth point (from the knee) and the midpoint of the knee-ankle distance of approximately 30–35 mm. These needles had a diameter of 0.2 mm (CE0123, Seirin-Kasei, Shimizu City, Japan) were inserted to a depth of ~10 mm in the skin and underlying muscle (the right tibialis anterior muscle). This area corresponds to the Zusanli and Xiajuxu acupoints (over the peroneal nerve below the knee, stomach meridian, St 36 and 39) in humans.

As in previous studies (2, 3, 17, 42), the stimulus current intensity was determined as 10 times of twitch threshold, which is the minimal electrical current required for eliciting visible muscle twitches of the stimulated leg. Actually, the current was 4.8 ± 0.3 mA (4.2–5.4 mA). An electric rectangular wave current with a frequency of 1 Hz and with pulse duration of 5 ms was passed between these two needles by using an electrical stimulator (SEN-7203, Nihon Kohden) except protocol 4 where shorter pulse durations were challenged.

Protocols

The experimental protocol was approved by the Animal Experimental Committee of National Cardiovascular Center Research Institute.

Protocol 1: effect of Zusanli electroacupuncture on AP, SNA, and baroreflex. Protocol 1-1 (BAROREFLEX CLOSED-LOOP CONDITION, N = 6). To elucidate the overall cardiovascular inhibitory effects of electroacupuncture, we performed 1 Hz electroacupuncture for 8 min and measured AP and SNA responses under conditions of intact cardiovascular reflexes. In this closed-loop protocol, vagal and aortic depressor nerves were preserved. Baseline data were measured for 1 min before acupuncture insertion. At 10 min after acupuncture insertion, baseline data were measured again for 1 min. Electroacupuncture was applied for 8 min. The recovery data were measured for 2 min after the cessation of electroacupuncture.

Protocol 1-2 (BAROREFLEX OPEN-LOOP CONDITION, N = 8). To elucidate the effects of electroacupuncture on the arterial baroreflex over an entire operating range, we performed a baroreflex-open loop experiment as follows. CSP was first decreased to 40 mmHg. After attainment of a steady state, CSP was increased from 40 to 160 mmHg in increments of 20 mmHg. Each pressure step was maintained for 60 s. We measured AP and SNA during the stepwise increase in CSP. Two trials (control and electroacupuncture trials) were performed on each rabbit. The order of the trials was randomized. The electroacupuncture trial was identical to the control trial except that electroacupuncture was commenced 1 min before the initiation of stepwise increase in CSP.

Protocol 1-3 (BAROREFLEX OPEN-LOOP CONDITION WITH PERNEAVER DENERVATION, N = 6). To identify the afferent pathway of electroacupuncture, we examined the effects of 1 Hz electroacupuncture on the arterial baroreflex after severing the right peroneal nerve at the level of the knee joint. Estimation of the baroreflex equilibrium diagram was conducted as in protocol 1-2 in the control and electroacupuncture trials. Four of the six rabbits had also undergone protocol 1-2.

Protocol 2: effects of sham (nonelectrical) acupuncture at Zusanli and control (nonspecific) electrical and nonelectrical acupunctures on AP and SNA in baroreflex closed-loop condition (n = 6). To determine whether changes in AP and SNA during Zusanli electroacupuncture are specific responses, sham and control acupunctures were conducted under the following acupuncture conditions: 1) no acupuncture (nonacupuncture), 2) nonelectrical acupuncture at Zusanli-Xiajuxu (St 36–39) acupoints (sham acupuncture), 3) nonelectrical acupuncture at Guangming-Xuanzhong ( gallbladder meridian, Gb 37–39) acupoints (control acupuncture), and 4) electrical acupuncture at Guangming-Xuanzhong acupoints (control electroacupuncture). We chose Guangming-Xuanzhong as nonspecific control acupoints (trials 3 and 4) because these acupoints are believed to reduce leg pain without affecting the cardiovascular system, in contrast to the Zusanli-Xiajuxu acupoints. In each trial, AP and SNA were measured for a baseline duration of 1 min, under acupuncture condition (trial 1, 2, 3, or 4) for 8 min, and recovery for 1 min.

Protocol 3: effect of long-term Zusanli electroacupuncture on AP and SNA in baroreflex closed-loop condition (n = 6). To clarify the effect of long-term electroacupuncture on cardiovascular system, AP and SNA were measured during and after 30 min of electroacupuncture at Zusanli-Xiajuxu acupoints. Protocol 3 was conducted in the same manner as protocol 1-1 except with a longer stimulation duration than protocol 1-1 (8 min).

Protocol 4: Effect of pulse duration of Zusanli electroacupuncture on AP and SNA in baroreflex closed-loop condition (n = 6). To examine the effect of pulse duration of electroacupuncture on AP and SNA, AP and SNA were measured during electroacupuncture at Zusanli-Xiajuxu acupoints with the pulse duration increasing stepwise from 0.1 to 0.25, 0.5, 1, 2.5, 5, and 10 ms, every 60 s. In each animal, the frequency and stimulus current intensity were maintained constant as in protocols 1, 2, and 3.

Data Analysis

We recorded CSP, SNA, and AP at a sampling rate of 200 Hz by using a 12-bit analog-to-digital converter. Data were stored on the hard drive of a dedicated laboratory computer system for later analyses.

In protocol 1-1, 2, and 4, mean AP and SNA for 1 min were calculated for baseline conditions, every minute of electroacupuncture, and recovery. In protocol 3, mean AP and SNA for 5 min were calculated for baseline conditions, electroacupuncture, and recovery. In protocols 1-2 and 1-3, we calculated mean AP and SNA during the last 10 s of each CSP step. Because the absolute magnitude of SNA depended on recording conditions, SNA was presented in arbitrary units (au). The background noise level was set at 0 au and the SNA value at the closed-loop operating point in the control trial (without electroacupuncture) was set at 100 au for each animal.

A four-parameter logistic function analysis was performed on the neural arc (CSP-SNA data pairs) and the peripheral arc (SNA-AP data pairs) as follows (11)
where \( x \) and \( y \) represent the input and the output, respectively. \( P_1 \) denotes the response range (i.e., the difference between the maximum and minimum values of \( y \)), \( P_2 \) is the coefficient of gain, \( P_3 \) is the midpoint of the logistic function on the input axis, and \( P_4 \) is the minimum value of \( y \). The maximum gain \( (G_{max}) \) is calculated from \(-P_1 P_2/4\) at \( x = P_3 \). The parameter values were calculated by an iterative nonlinear least-squares regression known as the downhill simplex method.

**Statistical Analysis**

All data are presented as means ± SD. Differences were considered to be significant when \( P < 0.05 \). In protocols 1-1, 2, 3, and 4, the effects of electroacupuncture on AP and SNA at different time intervals were evaluated by one-way ANOVA. The Dunnett’s test was used for multiple comparisons. In protocols 1-2 and 1-3, the effects of electroacupuncture on the four parameters of the logistic functions relating to the neural and peripheral arcs, as well as on the closed-loop operating point, were examined by using a paired \( t \)-test.

**RESULTS**

Figure 1A (protocol 1-1) shows a typical time series of AP and SNA in response to Zusanli-Xiajuxu electroacupuncture with intact cardiovascular reflexes. AP and SNA were reduced immediately after beginning electroacupuncture, and these remained reduced during 8-min electroacupuncture. Figure 1B illustrates the group-averaged AP and SNA in response to electroacupuncture. AP and SNA for baseline were unchanged by acupuncture insertion alone, while these values for 8-min electroacupuncture remained decreased from baseline. These values returned to baseline level after the cessation of electroacupuncture.

Figure 2 (protocol 1-2) shows a typical AP and SNA response to the increments in CSP in the control (Fig. 2, left) and electroacupuncture (Fig. 2, right) trials. A stepwise increase in CSP decreased SNA and AP in both trials. In the electroacupuncture trial, the AP and SNA response ranges to CSP decreased SNA and AP in both trials. The neural arc showed a more linear relationship between SNA and AP than the neural arc. In the peripheral arc, electroacupuncture did not affect any of the four parameters or \( G_{max} \) (Table 1 and Fig. 3B). The operating point determined by the intersection of the neural and peripheral arcs was moved toward lower AP and SNA from point \( a \) to point \( b \) by electroacupuncture (Fig. 3C and Table 1).

Figure 4 (protocol 1-3) shows the averaged baroreflex neural (Fig. 4A) and peripheral arcs (Fig. 4B) in control and electroacupuncture trials with severance of the peroneal nerve innervating the tibialis anterior muscle. Two arcs obtained in both trials were nearly superimposable. The four parameters and \( G_{max} \) in the neural and peripheral arcs and operating point were

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**Fig. 1.** Typical time series of arterial pressure (AP) and sympathetic nerve activity (SNA) during 8 min of 1-Hz electroacupuncture (A) and the averaged (\( n = 6 \)) AP and SNA (B) in protocol 1-1. Data include periods of baseline (Base, 1 min), electroacupuncture (8 min), and recovery (Rec, 1 min). Each data point represents average values over 1 min. *P < 0.05: significantly different from baseline after acupuncture insertion.* au, Arbitrary units.
not affected by electroacupuncture when the peroneal nerve was denervated (Table 2 and Fig. 4C).

Figure 5 (protocol 2) shows the changes in AP and SNA during nonacupuncture (without acupuncture), sham acupuncture [nonelectrical acupuncture at Zusanli-Xiajuxu (St 36–39)], control acupuncture [nonelectrical acupuncture at Guangming-Xuanzhong (Gb 37–39)] and control electroacupuncture (electrical acupuncture at Gb 37–39) trials. AP and SNA did not change in these trials.

Figure 6, A and B (protocol 3), shows a typical time series and the averaged data, respectively, of AP and SNA in response to long-term Zusanli-Xiajuxu electroacupuncture. AP and SNA decreased immediately after electroacupuncture was started and remained reduced during 30-min electroacupuncture. In addition, AP and SNA returned to the preelectroacupuncture baseline levels immediately after cessation of electroacupuncture.

Figure 7, A and B (protocol 4), shows a typical time series and the averaged data, respectively, of AP and SNA during Zusanli-Xiajuxu electroacupuncture with the pulse duration increasing from 0.1 to 5 ms. Although increasing the pulse duration from 0.1 to 1 ms did not change AP and SNA, pulse durations of 2.5 ms and higher decreased SNA while pulse durations of 5 and 10 ms decreased AP.

Table 1. Effect of electroacupuncture on the operating point of baroreflex and on the 4 parameters of logistic functions approximating neural and peripheral baroreflex arcs

<table>
<thead>
<tr>
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<th>Control</th>
<th>Electroacupuncture</th>
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<tr>
<td>Operating point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial pressure, mmHg</td>
<td>108.4±8.7</td>
<td>98.8±7.9†</td>
</tr>
<tr>
<td>Sympathetic nerve activity, au</td>
<td>99.8±4.1</td>
<td>80.0±8.9†</td>
</tr>
<tr>
<td>Neural arc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P_1), au</td>
<td>144.0±35.0</td>
<td>112.6±9.2†</td>
</tr>
<tr>
<td>(P_2), au/mmHg</td>
<td>0.08±0.03</td>
<td>0.09±0.09</td>
</tr>
<tr>
<td>(P_3), mmHg</td>
<td>111.4±6.5</td>
<td>103.3±10.0*</td>
</tr>
<tr>
<td>(P_4), au</td>
<td>17.5±6.1</td>
<td>17.4±8.7</td>
</tr>
<tr>
<td>G_{max}, au/mmHg</td>
<td>-2.94±0.91</td>
<td>-2.58±1.27</td>
</tr>
<tr>
<td>Peripheral arc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P_1), mmHg</td>
<td>129.6±20.5</td>
<td>125.9±19.5</td>
</tr>
<tr>
<td>(P_2), au/mmHg</td>
<td>-0.03±0.01</td>
<td>-0.03±0.01</td>
</tr>
<tr>
<td>(P_3), au</td>
<td>80.6±23.2</td>
<td>71.7±17.1</td>
</tr>
<tr>
<td>(P_4), mmHg</td>
<td>29.9±16.3</td>
<td>29.5±12.1</td>
</tr>
<tr>
<td>G_{max}, mmHg/au</td>
<td>0.74±0.10</td>
<td>0.84±0.18</td>
</tr>
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</table>

Values are means ± SD (n = 8). G_{max}, maximum gain. See Data Analysis for definition of 4 parameters of logistic function. au, Arbitrary units. †P < 0.05 and *P < 0.005 vs. control.

DISCUSSION

The major new finding of the present study was that electroacupuncture at Zusanli resets the arterial baroreflex neural arc to lower SNA but does not significantly affect the baroreflex peripheral arc. As a result, the operating point determined by the intersection of the neural and peripheral arcs was moved toward lower SNA and AP by electroacupuncture. To the best of our knowledge, this is the first study delineating the effects of short-term electroacupuncture on the arterial baroreflex over an entire operating range.

Effects of Electroacupuncture on the Arterial Baroreflex (Protocol 1)

The arterial baroreflex system is one of the most important negative-feedback systems that stabilize AP against exogenous disturbances. When AP is decreased by exogenous perturbation such as blood loss, the reduction in AP is sensed by the arterial baroreceptors. SNA is then increased by the arterial baroreflex to buffer the reduction in AP. In such circumstances, SNA and AP change reciprocally. On the other hand, when SNA is changed by an exogenous perturbation such as emotional stress, SNA and AP change reciprocally. In protocol 1-1, electroacupuncture decreased both SNA and AP, indicating that electroacupuncture introduced exogenous perturbation to decrease SNA with a resultant reduction in AP. Although the net effect of electroacupuncture is to decrease SNA, the perturbation of AP cannot be excluded. For example, because electroacupuncture also twitched the hindlimb muscles, electroacupuncture might have perturbed AP via changes in vascular resistance and/or venous return through muscle pump function. Therefore, to quantify the contribution of both perturbations on SNA and on AP, we performed protocol 1-2. Perturbation of AP is most easily detected by comparing AP at the same SNA level with and without electroacupuncture.

In protocol 1-2, we performed a baroreflex open-loop experiment and identified the static characteristics of the neural and peripheral arcs over a wide operating range. As expected, electroacupuncture shifted the neural arc toward lower SNA and decreased maximum SNA to ~80% of control (Fig. 3A). This shift is not due to reduced perfusion to the medulla by AP reduction during electroacupuncture because the AP was decreased by ~10 mmHg and would not induce cerebral ischemia. In contrast, electroacupuncture had little effect on the peripheral arc (Fig. 3B). In other words, AP with and without electroacupuncture did not differ significantly at any of the SNA levels. Therefore, changes in AP observed in protocol 1-1...
were attributable exclusively to perturbation of SNA and not to possible perturbation effects of electroacupuncture on AP.

The neural and peripheral arcs were combined to yield a baroreflex equilibrium diagram (Fig. 3C). The closed-loop operating point, determined by the intersection of the neural and peripheral arcs, moved from point a to point b during electroacupuncture. Despite a significant shift in the closed-loop operating point, neither the neural nor peripheral arc gain was altered significantly (Table 1). The fact that the baroreflex gain was maintained during electroacupuncture suggests the possible application of electroacupuncture to the treatment of cardiovascular diseases with sympathetic hyperactivity. However, the preservation of the arterial baroreflex gain in the present experimental settings may rely on normal peripheral arc characteristics. Cardiovascular diseases such as heart failure may decrease the peripheral arc gain to a variable extent due to impaired pump function. Whether the arterial baroreflex function during electroacupuncture can be maintained in cardiovascular diseases awaits future study.

Mechanisms for the Cardiovascular Inhibitory Effects of Electroacupuncture (Protocol 1)

The resetting in the baroreflex neural arc during electroacupuncture was mediated by a somatosympathetic reflex arising from the stimulated hindlimb, as evidenced by the fact that peroneal denervation abolished the resetting (Table 2 and Fig. 4). This result was consistent with an earlier study (27) showing that depressor and sympathoinhibitory responses during acupuncture were abolished by sciatic and femoral denervation. The existence of a somatosympathetic reflex is also supported by the fact that electrical stimulation of somatic afferents reduced AP (7–9). Legramante et al. (14) showed that rapidly conducting group III somatic afferent activation can evoke AP reduction during 1-Hz electrical stimulation of the tibial nerve. In contrast, high-frequency stimulation of the somatic afferent evokes AP elevation. Passive muscle stretching, which is considered to activate group III somatic afferent fibers, shifts the baroreflex neural arc toward higher SNA, resulting in an increase in the closed-loop operating point (41). The mechanism of two opposing influences of somatic afferent activation depending on the stimulation frequency is not fully understood.

### Table 2. Effect of electroacupuncture with peroneal denervation on the operating point of baroreflex and on the 4 parameters of logistic functions approximating neural and peripheral baroreflex arcs

<table>
<thead>
<tr>
<th>Operating point</th>
<th>Control</th>
<th>Electroacupuncture</th>
</tr>
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<tbody>
<tr>
<td>Arterial pressure, mmHg</td>
<td>105.7 ± 5.7</td>
<td>104.1 ± 5.6</td>
</tr>
<tr>
<td>Sympathetic nerve activity, au</td>
<td>99.8 ± 5.1</td>
<td>98.3 ± 11.1</td>
</tr>
<tr>
<td>Neural arc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_1$, au</td>
<td>138.3 ± 42.4</td>
<td>136.3 ± 38.6</td>
</tr>
<tr>
<td>$P_2$, au/mmHg</td>
<td>0.11 ± 0.03</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>$P_3$, mmHg</td>
<td>112.7 ± 10.2</td>
<td>111.5 ± 10.6</td>
</tr>
<tr>
<td>$P_4$, au</td>
<td>14.9 ± 8.7</td>
<td>15.7 ± 7.4</td>
</tr>
<tr>
<td>$G_{max}$, au/mmHg</td>
<td>−3.27 ± 1.15</td>
<td>−2.84 ± 1.12</td>
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<tr>
<td>Peripheral arc</td>
<td></td>
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</tr>
<tr>
<td>$P_1$, mmHg</td>
<td>144.1 ± 35.5</td>
<td>140.5 ± 34.4</td>
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<tr>
<td>$P_2$, au/mmHg</td>
<td>−0.02 ± 0.002</td>
<td>−0.02 ± 0.004</td>
</tr>
<tr>
<td>$P_3$, au</td>
<td>82.0 ± 34.0</td>
<td>78.8 ± 32.0</td>
</tr>
<tr>
<td>$P_4$, mmHg</td>
<td>26.1 ± 8.1</td>
<td>25.5 ± 5.3</td>
</tr>
<tr>
<td>$G_{max}$, mmHg/au</td>
<td>0.69 ± 0.13</td>
<td>0.72 ± 0.21</td>
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</tbody>
</table>

Values are means ± SD (n = 6). See Data Analysis for definition of 4 parameters of logistic function.
Another explanation for resetting in the neural arc may be the release of endogenous opioids (e.g., β-endorphin and enkephalin), which are released from the adrenal gland and hypothalamus by prolonged (>30 min) electroacupuncture (20, 21). These endogenous opioids are known to modulate the arterial baroreflex (24, 29, 35). However, changes in endogenous opioids are unlikely to be the mechanism for reductions in SNA and AP by electroacupuncture in the present experimental settings because the inhibitory effects terminated immediately after cessation of electroacupuncture rather than lasting for several hours (42) (Fig. 1).

Previous studies suggest a central interaction between an electroacupuncture-evoked somatosympathetic reflex and the arterial baroreflex. Baroreceptor afferent inputs inhibit neural activities in the rostral ventrolateral medulla (rVLM) (6, 33). Tjen-A-Looi et al. (36) showed that electroacupuncture inhibited rVLM neural activities, suggesting that the electroacupuncture-evoked somatosympathetic reflex and arterial baroreflex share common central pathways. In addition, 2-Hz electroacupuncture inhibits SNA through the excitation of β-endorphinergic and GABAergic neurons to rVLM (12, 13).

Central interaction in the brain stem may be involved in the resetting of the arterial baroreflex neural arc induced by electroacupuncture.

Characteristics of Zusanli-Xiajuxu Electroacupuncture Used in the Present Study

The Zusanli electroacupuncture used in this study has some unique characteristics. First, our results showed that baseline AP and SNA were decreased significantly by electroacupuncture, in contrast to previous studies that found no significant reduction in baseline AP and SNA during Zusanli electroacupuncture in rats (0.5-ms duration, 1–2 mA, 2 Hz) (18) and nonelectrical acupuncture in normotensive humans (right large intestine 4, right liver 3, and left spleen 6) (22). Second, our result showed that AP and SNA were reduced as soon as electroacupuncture was started, in contrast to previous reports that the effect of Zusanli electroacupuncture did not even begin to manifest for the first 10–15 min in rats (0.5-ms duration, 1–2 mA, 2 Hz) (18) and cats (0.5-ms duration, 0.4–0.6 mA, 2–4 Hz) (37). These discrepancies may be related to the differences in acupoints and stimulation conditions (pulse duration, current, and frequency). In particular, the pulse duration used in our study (5 ms) was approximately 10–50 times longer than that used in previous studies. Indeed, the data obtained from protocol 4 show that increasing the pulse duration augments the reduction in AP and SNA during electroacupuncture; pulse durations shorter than 2.5 ms did not change AP and SNA, whereas durations of 2.5 ms and above decreased both parameters immediately after the electroacupuncture was started (Fig. 7). In addition, our data suggest that stimulation duration (<2.5 ms) does not affect arterial baroreflex, consistent with our preliminary data that baroreflex neural, peripheral, and total arcs remained unchanged during electroacupuncture with pulse durations <2.5 ms (unpublished data). These observations may indicate that the effect of electroacupuncture on arterial baroreflex is linked to the stimulation pulse duration.

The third characteristic is that the inhibitory effects of electroacupuncture on AP and SNA disappeared immediately after the cessation of electroacupuncture. In contrast, some studies showed that the inhibitory effects of electroacupuncture on AP lasted for 10–60 min after the cessation (18). The characteristics in this study may not be explained by the length of electroacupuncture because AP and SNA recovered to the

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Fig. 6. Typical time series of AP and SNA during 30 min of 1-Hz electroacupuncture (St 36–39; A) and the averaged (n = 6) AP and SNA (B) in protocol 3. Data include periods of baseline (5 min), electroacupuncture (30 min), and recovery (30 min). Each data point represents averaged values over 5 min during baseline, electroacupuncture, and the first 10 min of recovery and those over 10 min during the last 20 min of recovery. *P < 0.05: significantly different from baseline after acupuncture insertion.

Fig. 7. Typical time series of AP and SNA during 1-Hz electroacupuncture with increasing the pulse duration (A) and the averaged (n = 6) AP and SNA (B) in protocol 4. Data include periods of baseline (B, 1 min), electroacupuncture (7 min), and recovery (R, 1 min). Each data point represents average values over 1 min. *P < 0.05; significantly different from baseline after acupuncture insertion.
prestimulation baseline levels immediately after the cessation in both short-duration (8 min, Fig. 1) and longer-duration electroacupuncture (30 min, Fig. 6) protocols. The rapid disappearance of effects suggests that the AP and SNA reductions seen in the present study may not be elicited by the opioid mechanism, although clinical experiments with longer-duration electroacupuncture have demonstrated long-lasting effects on the cardiovascular system, which are attributed to opioid substances (2, 12, 15, 37, 42).

The reductions in AP and SNA during Zusanli electroacupuncture seen in the present study may not be a nonspecific response to acupunctures. Our data from protocol 2 (Fig. 5) showed that nonelectrical acupuncture at Zusanli (sham acupuncture) did not decrease AP and SNA, suggesting that the AP and SNA reductions during Zusanli electroacupuncture are not simply the results from insertion of acupuncture needles. Furthermore, acupuncture at Guangming-Xuanzhong (control acupuncture, control electroacupuncture) did not change AP and SNA regardless of electrical stimulation (Fig. 5). This result suggests the importance of acupoint specificity and is consistent with an earlier study showing point-specific differences in cardiovascular inhibitory responses (Jiangshi-Neiguan or Shousanli-Quchi acupoints vs. Pianli-Wenlui or Zusanli-Shangjuxu acupoints) (37). These observations may support the concept that Zusanli acupuncture changes cardiovascular variables in experimental animal models (4, 25, 28) and confers beneficial effects on cardiovascular diseases (5, 30, 34), whereas Guangming-Xuanzhong acupuncture does not affect cardiovascular variables (18).

Limitations

There are several limitations to this study. First, as anesthesia affects the autonomic nervous system, the results might have been different without anesthesia. Second, our isolation of the carotid sinus regions may stimulate carotid chemoreceptors. However, in determining baroreflex function, this factor was present in trials with and without electroacupuncture. Therefore, we believe that this factor may not affect our conclusion of baroreflex resetting during electroacupuncture.

Third, acupuncture was inserted at a point corresponding to the Zusanli acupoint in humans. When acupuncture is properly inserted at the acupoint, the patient feels heaviness or soreness. Such sensory information is not available in an anesthetized animal. Because electroacupuncture (as distinct from acupuncture with no electrical stimulation) stimulates not only the inserted point but also the surrounding area, it has been used as a convenient way of stimulating acupoints in patients and in experimental animals. Thus, even if we failed to insert the needle at the precise acupoint, we believe that Zusanli could be stimulated electrically.

Fourth, although we determined the effects of electroacupuncture at Zusanli acupoints on cardiovascular and baroreflex systems, there are other important acupoints that are able to influence these systems. In particular, Neiguan electroacupuncture is actually known to decrease sympathetic premotor neuron activity for a longer period than Zusanli electroacupuncture (36, 37). Further studies are necessary to determine the effect of Neiguan electroacupuncture on the arterial baroreflex.

Last, we evaluated the effects of Zusanli electroacupuncture on the baroreflex function for a short acupuncture duration of only 8 min. Because electroacupuncture is typically practiced for longer periods of time, our results have limited applicability. However, the electroacupuncture we used decreased AP and SNA immediately after application, showing that the procedure has acute effect on the cardiovascular system. That was the reason why we focused on the effect of short duration electroacupuncture on the baroreflex system. Future study is necessary to examine the effects of longer-duration electroacupuncture.

In conclusion, 1 Hz, short-term electroacupuncture of Zusanli reset the baroreflex neural arc toward lower SNA but did not affect the peripheral arc. The closed-loop operating point determined by the intersection of the neural and peripheral arcs was moved toward lower SNA and AP by electroacupuncture.

APPENDIX

Theoretical Considerations: Coupling of Neural and Peripheral Arcs

Changes in AP are immediately sensed by arterial baroreceptors, which alter efferent SNA via the cardiovascular center of baroreflex (Fig. 8A). Efferent SNA in turn governs heart rate and the mechanical
properties of the heart and vessels, which themselves exert a direct influence over AP. This negative-feedback loop makes it difficult to analyze the behavior of the arterial baroreflex. To overcome this problem, we opened the negative-feedback loop and divided the system into controlling and controlled elements (31). We defined the controlling element as the neural arc and the controlled element as the peripheral arc (Fig. 8B). In the neural arc, the input is the pressure sensed by the arterial baroreceptors and the output is SNA. In the peripheral arc, the input is SNA and the output is AP (Fig. 8C).

Because pressure sensed by the arterial baroreceptor is equilibrated with AP under physiological conditions, we superimposed the functions of the two arcs and determined the operating point of the system from the intersection of the two arcs. The operating point is defined as the AP and SNA under closed-loop conditions of the feedback system. The validity of this framework has been examined in previous studies (10, 31). Using the baroreflex equilibrium diagram, we aimed to quantify the effects of electroacupuncture on the arterial baroreflex.

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