Neiguan (PC-6) is a traditional acupoint in the bilateral forearms, overlying the median nerve trunk. Neiguan electroacupuncture (EA) has been believed to affect cardiovascular function and used in traditional Chinese medicine to improve or treat a wide range of heart conditions and diseases, including angina pectoris, myocardial infarction, hypertension, and hypotension. However, few physiological studies have assessed the beneficial effects of Neiguan EA on the cardiovascular function. In the present study, we investigated its effects on the cardiovascular function in normal open-chest dogs under pentobarbital and fentanyl anesthesia. We also obtained left ventricular (LV) pressure-volume (P-V) data with a micromanometer catheter and a volumetric conductance catheter. Mean arterial pressure, end-diastolic volume, heart rate, stroke volume, cardiac output, and end-systolic pressure gradually decreased by 5 to 10% over 1.5 h without Neiguan EA. Neiguan EA at 40 Hz, however, increased these cardiovascular variables by 10 to 15%, especially end-systolic elastance (Ees) by 40% (p<0.05) over 15 to 60 min. After Neiguan EA was stopped at 1 h, these facilitated cardiovascular variables decreased below the pre-EA level. This beneficial effect of electroacupuncture may contribute to the effectiveness of the acupuncture in Chinese medicine.


**Abstract:** Neiguan (PC-6) is a traditional acupoint in the bilateral forearms, overlying the median nerve trunk. Acupuncture at some acupoints including Neiguan, has been believed to affect cardiovascular function and is used in traditional Chinese medicine to improve or treat a wide range of health conditions and diseases, including angina pectoris, myocardial infarction, hypertension, and hypotension. However, few physiological studies have assessed the beneficial effects of Neiguan EA on the cardiovascular function. In the present study, we investigated its effects on the cardiovascular function in normal open-chest dogs under pentobarbital and fentanyl anesthesia. We also obtained left ventricular (LV) pressure-volume (P-V) data with a micromanometer catheter and a volumetric conductance catheter. Mean arterial pressure, end-diastolic volume, heart rate, stroke volume, cardiac output, and end-systolic pressure gradually decreased by 5 to 10% over 1.5 h without Neiguan EA. Neiguan EA at 40 Hz, however, increased these cardiovascular variables by 10 to 15%, especially end-systolic elastance (Ees) by 40% (p<0.05) over 15 to 60 min. After Neiguan EA was stopped at 1 h, these facilitated cardiovascular variables decreased below the pre-EA level. This beneficial effect of electroacupuncture may contribute to the effectiveness of the acupuncture in Chinese medicine.

**Key words:** Chinese medicine, Neiguan acupoint, cardiac performance, ventricular contractility.

**METHODS**

**Surgical preparation.** Ten adult mongrel dogs...
(body weight 7 to 12 kg) were anesthetized with pentobarbital sodium (10 to 15 mg/kg, i.v.) after premedication with ketamine hydrochloride (7 mg/kg, i.m.) and intubated in each experiment. Anesthesia was maintained with pentobarbital (1 to 2 mg/kg/h, i.v.) and fentanyl (200 μg/h, i.v.). All experimental studies were performed according to the animal use guideline set by the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

We inserted a fluid-filled catheter in the right femoral artery for arterial pressure measurement. Another was positioned in the right atrium via the right internal jugular vein for hypertonic saline injection. We also cannulated the right femoral vein for administration of other fluids. Arterial blood gases and pH were maintained within the physiological range by administration of other fluids. Arterial blood gases and pH were maintained within the physiological range by administration of other fluids. Arterial blood gases and pH were maintained within the physiological range by administration of other fluids.

**Neiguan electroacupuncture.** Ten dogs were divided into a control group (N=4) and a Neiguan electroacupuncture (EA) group (N=6). In every dog, we inserted two stainless steel acupuncture needles (Suzhou Acupuncture Medical Appliance, Suzhou, PRC) vertically to a depth of 18 to 19 mm at Neiguan (PC-6) acupoints in bilateral forearms. This position was 3 cm above the transverse crease of the wrist and between the tendons of the long palmar muscle and the radial flexor muscle of the wrist.

We selected this needle positioning according to the previous study on the cat by other investigators [7]. The correct positioning of acupuncture needles in human subjects relies on their feeling of “heaviness” associated with electrical stimulation 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 had to rely on our observation of a slight repetitive flexion of the paw during electrical stimulation, as in previous study [7].

We electrically stimulated Neiguan acupoints at 40 Hz and 5 V with an EA stimulator (WQ-6F, Dong Hua Electronic Instrument Factory, Beijing, PRC) for 60 min. We had found 40 Hz and 5 V to be the most effective in our preliminary experiment. This stimulation caused visible local muscle tetanic contractions and slight repetitive paw flexion [7], by which we confirmed correct positioning at Neiguan acupoint.

**LV pressure and volume measurement.** We introduced a precalibrated 2.5-F micromanometer-tipped catheter (SPR-524, Millar Instruments, Inc., Houston, TX, USA) and an 8-F conductance catheter (custom made by Inter Medical, Osaka, Japan) into the left ventricle (LV) through the apex. We positioned the distal driving electrode of the conductance catheter at the level of the aortic valve and fixed the proximal one near the apex with an apical suture. We attached three epicardial electrodes for recording electrocardiogram (ECG) and placed a tape snare around the descending aorta for transient aortic occlusion. To minimize the effect of parallel conductance (i.e., conductance of tissues surrounding the LV cavity), we placed a piece of insulated rubber membrane around the heart.

The conductance catheter had eight platinum ring electrodes, of which the six inner electrodes (6.5 mm apart) sensed five segmental conductance (Gi, i=1–5) signals. The two outermost electrodes (electrode distance, 45.5 mm) delivered a constant high-frequency current (0.03 mA root mean square at 20 kHz) from a conductance signal–processing apparatus (custom made by S-I Medico-Tech Co., Ltd., Osaka, Japan). The circuit was designed to measure Gi with the six sensing electrodes. The summed conductance Gi=ΣGi (i=1–5) is theoretically proportional to the LV blood volume (LVV) [8]. The conductance catheter method of measuring LV volume (LVV) was described in detail elsewhere [8, 9]. Briefly, we obtained instantaneous intraventricular conductance volume V(t) from the measured conductance G(t) by the following equation.

\[
V(t) = (1/\alpha) \times \rho \times L_2 \times [G(t) - G_p] \\
= (1/\alpha) \times \rho \times L_2 \times G(t) - V_c \tag{1}
\]

\[
V_c = (1/\alpha) \times \rho \times L_2 \times G_p \tag{2}
\]

where α is a dimensionless empirical constant for the V(t)-G(t) relation. This value was reasonably assumed to be 1.0 [8]. L is the distance across the six sensing electrodes (6.5×5=32.5 mm). μ is the specific resistivity of blood. We measured ρ with a blood resistivity curette (S-I Medico-Tech Co., Ltd.). Gp is the parallel conductance, which partially conducts the driving current and overestimates intraventricular G(t). To obtain V(t), we had to determine Gp by the hypertonic saline (20% NaCl) dilution method [8, 9]. We calculated the constant offset volume (Vc) corresponding to Gp by Eq. 2. The calculated Vc value was subtracted from V(t) to obtain the absolute LVV. At the end of each experiment, the LV including the interventricular septum was excised and weighed after both the atria and the right ventricular free wall were removed. The LV weight was used to normalize LVV for 100 g of LV mass.
After measuring the \( r \) value, we injected 2 ml of 20% hypertonic saline into the right atrium and repeated this measurement three times to determine \( V_c \) in each experiment.

**Data analysis.** ECG, LV pressure, and segmental conductance volume (\( G_t \)) signals as well as the other hemodynamic variables were digitized with an analog-to-digital converter (Lab Nb, National Instruments Corp., Austin, TX, USA) at a sampling frequency of 500 Hz, and stored in a computer (Power Macintosh 8100/100AV, Apple Computer, Inc., Cupertino, CA, USA). To exclude the respiratory changes in hemodynamics, we stopped artificial ventilation during data recording.

We analyzed the pressure-volume (\( P-V \)) data obtained during a transient aortic occlusion, using our original software designed with Lab-VIEW 3.1\(^\circ\) (National Instruments Corp.). LV end-systolic elastance (\( E_{es} \)) was obtained by drawing a linear enveloping line on several \( P-V \) loops during the aortic occlusion. This index has been conventionally used to assess LV contractility [10]. \( E_{es} \) is advantageous over other contractility indices, since it is relatively independent of LV preload and afterload [10].

We obtained mean arterial pressure (MAP) by averaging arterial blood pressure with a low-pass filter having a time constant of 2 s. We also obtained heart rate (HR) from ECG and stroke volume (SV) as the width of the \( P-V \) loop. Cardiac output (CO) was obtained as the product of HR and SV. Total peripheral resistance (TPR) was obtained by dividing CO with MAP.

**Experimental protocol.** In the control group, we first obtained all the cardiodynamic data as soon as the data acquisition became ready. These data were basal. The same data acquisition was repeated every 15 min for 90 min in each experiment with no intentional interventions.

In the Neiguan EA group, we first obtained all the cardiodynamic data as soon as the surgical preparation was over. These data were also basal. We then started Neiguan EA and continued it for the first 60 min, then stopping it for the next 30 min. The same data acquisition was repeated every 15 min for 90 min in each experiment.

**Statistics.** All values are presented as mean±SD. \( p<0.05 \) was used for significance in all statistical tests. The effects of Neiguan EA at each time point were analyzed by using ANOVA with a repeated measure design (Statview v.5.0, Abacus Concepts, Berkeley, CA, USA). When significant differences between control and Neiguan EA groups were detected by ANOVA, nonpaired \( t \)-tests with Bonferroni’s correction for multiple comparisons were performed to determine which individual differences were statistically significant. Dunnett’s test compared each time point during EA and after EA with the baseline value.

**RESULTS**

Figure 1 shows a representative set of steady-state \( P-V \) loops of the *in situ* canine LV at basal and 60 min in one control experiment (Fig. 1A) and one Neiguan EA experiment (Fig. 1B). End-systolic pressure (ESP), end-diastolic volume (EDV), and SV decreased at 60 min in control heart, indicating a decreased LV contractility. On the contrary, Neiguan EA increased ESP and shifted the \( P-V \) loop to the left from the basal loop, indicating an increased LV contractility. Similar changes were more or less observed in the other hearts of control as well as in the Neiguan EA group.

Figure 2 shows representative sets of \( P-V \) loops obtained during a transient aortic occlusion at basal (0 min, Fig. 2, A and C) and 60 min without (control, Fig. 2B) and with Neiguan EA (Fig. 2D). A linear regression line was drawn through the end-systolic \( P-V \) points during the aortic occlusion to obtain LV end-systolic elastance (\( E_{es} \)) and volume-axis intercept (\( V_0 \)).
in each case (see inset). $V_0$ changed only slightly over time, and $E_{es}$ decreased only slightly over time in the control case (Fig. 2, A and B). In contrast, $E_{es}$ increased considerably in the Neiguan EA case, where $V_0$ decreased slightly. The other control and Neiguan EA cases showed more or less similar results to these representative cases.

Table 1 lists mean±SD of all the measured hemodynamic variables from 0 (basal) to 90 min at 15 min intervals in both control and Neiguan EA groups. Figure 3 illustrates the percent changes of these variables relative to the respective basal levels.

**Fig. 2.** The changes in $E_{es}$ at basal (A and C) and 60 min (B and D) in control (A and B) and Neiguan EA (C and D). End-systolic $P$-$V$ points were fitted by a straight line to obtain $E_{es}$ as its slope. $V_0$ is LV volume at which LV end-systolic pressure was 0 mmHg. $E_{es}$ and LV volume were normalized to 100 g of LV mass.
Mean arterial pressure

In the control group, the mean arterial pressure (MAP) gradually decreased from its basal level over 1 h, then plateaued until 90 min. In the Neiguan EA group, MAP increased under the 1-h EA from its pre-EA basal level, but decreased toward the basal level after the EA was stopped (Fig. 3A and Table 1). MAP in the Neiguan EA group was significantly higher than that in the control group (*) over 30 to 90 min and also significantly (#) higher at 1 h than the basal level.

End-diastolic volume

End-diastolic volume (EDV) in the control group gradually decreased over 1.5 h and was significantly (#) lower than the basal level over 45 to 90 min. However, EDV in the Neiguan EA group was maintained...
at the basal level until 60 min and significantly (*) higher than that in the control group (Fig. 3B and Table 1). After Neiguan EA was stopped, EDV decreased below the basal level toward the control level.

**Heart rate**
Heart rate (HR) in the control group decreased gradually over 30 to 90 min. HR in the Neiguan EA group remained significantly (*) higher than that in the control group over 30 to 60 min (Fig. 3C and Table 1). After the EA, HR decreased below the basal level.

**Stroke volume**
Stroke volume (SV) in the control group decreased gradually over 1.5 h. Neiguan EA, however, slightly increased SV from the basal level (Fig. 3D and Table 1). After the EA was stopped, SV decreased below the basal level toward that in the control group.

**Cardiac output**
Cardiac output (CO) behaved in a manner similar to SV in both the control and the Neiguan EA groups (Fig. 3E and Table 1).

**Total peripheral resistance**
Total peripheral resistance (TPR) increased gradually over 1 to 1.5 h in both the control and the Neiguan EA groups. There was no significant difference in TPR at corresponding times between the two groups (Fig. 3F and Table 1).

**End-systolic pressure**
End-systolic pressure (ESP) in the control group decreased slightly over 1 to 1.5 h. However, ESP in the Neiguan EA group increased gradually over 1 h and decreased toward the basal level after EA was stopped (Fig. 3G and Table 1). The EA group’s ESP was significantly higher than the control group’s ESP (*) and the basal level (+).

**End-systolic elastance**
End-systolic elastance ($E_{es}$) decreased slightly in the control group. $E_{es}$, however, increased markedly in the Neiguan EA group (Fig. 3H and Table 1). The Neiguan EA group’s $E_{es}$ was much higher than the control $E_{es}$ (*) as well as the basal level (+) over 15 to 60 min and even after the EA was stopped. $V_0$ did not change significantly with time in either the control or the Neiguan EA group.

**DISCUSSION**
Recently, U.S. National Institutes of Health (NIH) announced a consensus statement on acupuncture that acupuncture therapy has been an essential component of the health care system in China for at least 2000 years [11]. The statement says that acupuncture has been believed to correct imbalance of the so-called “energy flow” through the 12 primary meridians. Using a variety of assessment techniques, acupuncture practitioners have tried to identify the nature of the imbalance and have selected appropriate acupoints to correct the imbalance from approximately 360 points along the meridians [12]. Since acupuncture therapy has been developed from empirical trials for 2000 years [11], scientific studies for endorsing its clinical effectiveness and benefit are mandatory.

In the present study, we selected the Neiguan (PC-6) because it is one of the primary acupoints used clinically in traditional Chinese medicine to treat various cardiovascular diseases [1–6]. Neiguan is also an acupoint commonly used to study the effects of EA in several different animal models of cardiovascular diseases [5, 6]. However, research on Neiguan EA, using an animal model in the absence of disease, is lacking. To the best of our knowledge, the present study is the first to have provided physiological evidence that Neiguan EA is effective in maintaining hemodynamics and cardiac contractility in anesthetized open-chest dogs.

We found in this study that cardiovascular function was gradually depressed over 1.5 h in the control group with no specific intervention, as indicated in the control group (Fig. 3 and Table 1). This occurred although we combined fentanyl anesthesia with pentobarbital anesthesia to minimize defense responses, including catecholamine release resulting from the surgical invasion [13]. Fentanyl anesthesia decreases heart rate and blood pressure by reducing sympathetic activity [13]. However, previous studies show that fentanyl anesthesia does not depress cardiac contractility assessed by $E_{es}$ and is beneficial to maintain stable cardiovascular function [14, 15]. We would consider the gradual decreases in the hemodynamic variables, except for cardiac contractility in the present study, to be due to the prolonged anesthesia with pentobarbital and fentanyl and artificial ventilation after thoracotomy. This result is consistent with those obtained by others in anesthetized open-chest dogs [16–18].

In the present study, we newly found that Neiguan EA continued for 1 h maintained EDV at the basal level, increased the other hemodynamic variables by 5 to 10% above the basal level, and especially enhanced cardiac contractility by approximately 40%. After Neiguan EA was stopped at 1 h, these increased variables decreased gradually over the following 30 min.
below the basal level toward the control level. No previous studies, using scientifically established methods, have reported such beneficial effects of Neiguan EA on the anesthesia-depressed cardiovascular function.

We could speculate that these beneficial effects on the cardiovascular conditions would primarily be due to the sympathetic tone activated by Neiguan EA. Three possible mechanisms are conceivable. The first would be a pain response, but we could exclude this possibility under fentanyl anesthesia. The second would be an autonomic reflex initiated by hypotension or hypovolemia. We could also exclude this possibility because of no hypotensive or hypovolemic state under Neiguan EA.

The third possibility would be a somato-sympathetic reflex [19, 20]. A stimulation of somatic nerves evoked pressor or depressor response, depending on the stimulus intensity, frequency, and activated fiber type [20]. Strong electrical stimulation of a quadriceps nerve at 5 to 25 times threshold for flexion reflex caused a pressor response and enhance the left ventricular contractility, whereas weaker stimulation at 2 to 4 times threshold caused a decrease in the heart rate and arterial pressure in open-chest dog [21]. A stimulation of somatic afferents from muscle at 5 Hz and 5 V caused a depressor effect, whereas stimulation at 40 Hz and 5 V caused a pressor response [22]. Systematic studies on the effectiveness of the various types of somatic afferents, as classified by fiber diameter, were conducted by Johansson [23]. The results indicated that the stimulation of myelinated fibers alone or myelinated and unmyelinated fibers together could cause a depressor response, but stimulation of unmyelinated fibers alone could cause a pressor response.

Neiguan acupoint is close to the median nerve containing both myelinated and unmyelinated fibers. Chao et al. [7] reported that stimulation at Neiguan activated these fibers in the median nerve and caused a depressor response at 4 Hz and 2 to 5 V in cats. In contrast, our present study has demonstrated that Neiguan EA at 40 Hz and 5 V not only increased hemodynamic variables, but also greatly enhanced left ventricular contractility (Fig. 3 and Table 1). These results are largely consistent with the studies mentioned above [21, 22]. We therefore speculated that Neiguan EA at 40 Hz and 5 V augmented sympathetic tone and improved the inhibited cardiovascular function under anesthesia, though we were unable to quantitate the degree of involvement of the median nerve during Neiguan EA in the present study. When we decreased the EA stimulation frequency to 5 Hz in a preliminary experiment, we observed neither depressor nor pressor response, unlike the previous finding by Chao et al. [7]. We had to increase the frequency to 30 to 70 Hz to reproduce any recognizable responses.

Concerning somatic reflex effects on cardiovascular responses, we cannot neglect the effects of anesthetics on the chronotropism. Somatic stimulation can induce a decrease in cardiac vagal efferent activity under anesthesia, because various anesthetics appear to maintain cardiovascular sympathetic efferent activity at supranormal level, but they strongly suppress cardiac vagal efferent activity [20]. In contrast, somatic stimulation will decrease heart rate in conscious humans because the contribution of the vagus nerve to the reflex arc remains normal [24]. In the present study, we observed cardiac acceleration reflex under Neiguan EA (Fig. 3C) in the anesthetized dogs; the same results have also been observed in anesthetized cats [25] and rats [26] with somatic stimulation. We would therefore consider that the reflex positive chronotropism in the present study would be due to the Neiguan EA–induced cardiac sympathetic reflex.

Although the aim of the present study was not to elucidate the mechanisms of the beneficial effects of Neiguan EA, the proven beneficial effect per se is invaluable. Since we first observed the beneficial effect of Neiguan EA, we adopted it to maintain mean arterial blood pressure of the metabolic support dog in our routine cross-circulation heart experiment [27]. The support dog is unthoracotomized, though anesthetized with pentobarbital and fentanyl. Until we adopted Neiguan EA, mean arterial pressure gradually decreased, and we had to maintain it by transfusing blood reserved from the donor heart dog or by infusing saline [27]. However, since we adopted Neiguan EA, mean arterial pressure usually remained stable above 90 mmHg over several hours with little need of blood transfusion (unpublished observation).

In conclusion, we observed the beneficial effects of Neiguan EA to provide stable cardiovascular function in a long-term anesthetized animal experiment. However, we cannot yet exclude some contribution of the median nerve stimulation in the beneficial effects of Neiguan EA. There also remains a possibility that the same or similar beneficial effects could be elicited by other acupoints, especially those overlying the median nerve. The present results warrant future studies to elucidate the mechanisms underlying the beneficial effects of the electroacupuncture in traditional Chinese medicine. We recommend that readers interested in the effects of acupuncture on cardiovascular function refer to our review written in Japanese, but with an English abstract [6].

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