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The Study of Electroacupuncture on Cerebral Blood Flow in Rats With and Without Cerebral Ischemia

Ching-Liang Hsieh,[*,§] Qwang-Yuen Chang,[¶] I-hsin Lin,[¶] Jaung-Geng Lin,[//]
Chung-Hsiang Liu,[†] Nou-Ying Tang[¶] and Hsien-Yuan Lane[‡]
[*]*Departments of Chinese Medicine*, [†]*Neurology and* [‡]*Psychiatry*
China Medical University Hospital, *Taichung*, *Taiwan*
[§]*Graduate Institute of Integration of Chinese and Western Medicine*, [¶]*School of Chinese Medicine*
[//]*Graduate Institute of Chinese Medical Science*, *College of Chinese Medicine*
China Medical University, *Taichung*, *Taiwan*

Abstract: Electroacupuncture (EA) is widely used to treat disorders of the nervous system, such as stroke. The aim of the present study was to investigate the effect of EA on cerebral blood flow (CBF) in cerebral ischemic rats. We developed an animal model of cerebral ischemia (CI) by occluding the blood flow of both common carotid arteries in Sprague-Dawley (SD) rats; 2 or 15 Hz EA was applied to both Zusanli acupoints. The levels of nitric oxide (NO) in the peripheral blood and amounts of calcitonin gene-related peptide (CGRP) in the cerebral cortex and thalamus were measured. In addition, L-N (G)-nitro arginine methyl ester (L-NAME) was used to measure the changes in CBF induced by EA in rats with and without CI. The results indicated that both 2 and 15 Hz EA increase the mean CBF in rats with and without CI. However, neither 2 nor 15 Hz EA induced changes in levels of NO in peripheral blood or changes in CGRP levels in cerebral cortex and thalamus. In addition, L-NAME did not change the increase in CBF. We concluded that both 2 and 15 Hz EA at both Zusanli acupoints induced the increase of CBF in rats with and without CI. Whether the effect of EA is related to NO or CGRP will be investigated in a future study.

Keywords: Electroacupuncture; Cerebral Blood Flow; Cerebral Ischemia; Nitric Oxide; Calcitonin Gene-Related Peptide.

Correspondence to: Dr. Hsien-Yuan Lane, No. 2, Yuh-Der Road, Department of Psychiatry, China Medical University Hospital, Taichung, Taiwan. Tel: (+886) 4-2206- 2121 (ext. 5855), Fax: (+886) 4-2236-1042, E-mail: hylane@pchome.com.tw

Introduction

A number of studies have shown that acupuncture produces an analgesia effect by stimulating the release of endorphins (Clement-Jones *et al*., 1980; Han *et al*., 1981; Ulett *et al*., 1998; Adams and Assefi, 2001). The effect of acupuncture has also been reported to block other pain stimuli from being transmitted to the brain (Melzack and Wall, 1965; Wall, 1978; Adams and Assefi, 2001). Because electroacupuncture (EA) produces a greater analgesia effect than manual acupuncture (Ulett *et al*., 1998), EA has been widely used to treat disorders of the nervous system. Different frequency of EA may mediate the analgesia effect via different neuronal circuits. For example, high-frequency EA has been reported to cause more caudal brain stem nuclei excitation, while low-frequency EA evoked diencephalic nuclei excitation for analgesia production (Lee and Beitz, 1993). Acupuncture may regulate gastrointestinal motility and secretion via a neural pathway (Li *et al*., 1992); it may also, via the brain opoid peptidergic system, inhibit blood pressure response (Wang *et al*., 1994). In addition, acupuncture has been shown to induce the activation of cardiac vagal activity and the suppression of cardiac sympathetic activity to reduce heart rate (Yao *et al*., 1982; Nishijo *et al*., 1997). Several reports have proposed that acupuncture is inhibition of sympathetic activities, which are favorable to its analgesic role (Cao *et al.*, 1983; Ernst and Lee, 1986).

In a severe hypoxia state, NO influences cerebral oxygen vasoreactivity (Berger and Von Kummer, 1998). The upregulation of endothelial nitric oxide synthase (eNOS) mRNA occurred 2 hours after non-occlusive common carotid artery thrombosis in rats (Danton *et al*., 2002). In addition, acupuncture has been reported to reverse the increase in neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) levels of the hippocampus region in rats with penicillin-induced epileptic seizure (Yang *et al*., 1999). Acupuncture can increase the cerebral blood flow velocity of both middle cerebral arteries (Bäcker *et al*., 2002), and EA increases cerebral blood flow (CBF) in the contralateral cerebral hemisphere cortex, and thalamus (Adams and Assefi, 2001). Electrical stimulation of muscle afferent induced the release of calcitonin gene-related peptide (CGRP) from sensitive nerve terminal to produce skeletal muscle vasodilation (Sato *et al*., 2000). Furthermore, it has been postulated that EA increases cutaneous blood flow in rats due to the effect of CGRP and substance P (Jansen *et al*., 1989).

The aim of the present study was to investigate the effect of EA on CBF in rats with or without cerebral ischemia (CI). We developed an animal model of CI by occluding the CBF of both carotid arteries in Sprague-Dawley (SD) rats. CBF was recorded by a Laser Doppler cerebral blood flow monitoring apparatus. The probe was placed on the right middle cerebral artery just at the immediate upper margin of the olfactory tract; 2 or 15 Hz EA was applied to both Zusanli acupoints. The levels of NO in the peripheral blood, and CGRP in the cerebral cortex and thalamus were measured. In addition, L-N (G)-nitro arginine methyl ester (L-NAME) was used to measure the changes in CBF induced by EA.

Materials and Methods

Animals

Adult male Sprague-Dawley (SD) rats, weighing 200–300 g, were housed in iron cages and maintained on a 12-hour light-dark cycle at 25ºC. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals.

Establishment of CI Animal Model

A total of 36 SD rats were used in the present study. The rats were anesthetized with an intraperitoneal injection (i.p.) of chloral hydrate (400 mg/kg). The rats were placed in a supine position, and right femoral artery and vein were exposed by a skin incision. PE-50 tubes were inserted into the femoral artery and vein. The PE-50 tube in the femoral artery was connected to a heart rate-blood pressure measuring apparatus (LE 5001 pressure meter, Panlab. S.L.L., Barcelona, Spain). The blood pressure and heart rate of the rat were monitored, and body temperature was maintained at 37 ± 0.5ºC with a heated pad throughout the experimental procedure. The common carotid arteries of the rats were exposed by a midline incision and the arteries were wrapped by a plastic line loop (0.1 mm in diameter) and a PE-50 tube (0.2 mm in diameter). Next, the head was fixed in a stereotactic apparatus in a prone position. The scalp was incised to create an anterior-posterior direction wound (1.5 cm in length) from the midpoint of the binaural line. A bone window 3.5 mm in diameter was made after the temporal muscles were separated and the temporal bone was exposed. The olfactory tract and right middle artery were thus clearly visible. The probe of a Laser Doppler cerebral blood flow monitoring apparatus (DRT4, Moor Instruments Inc. Wilmington, USA) was placed just at the immediate upper margin of the right olfactory tract. The markers for laser Doppler perfusion were monitored from 900 to 200 units when the loops of the plastic line were drawn to block the blood flow of the common carotid arteries. Each session of the experiment was 90 minutes; the systolic blood pressure was recorded at 0, 30, 60 and 90 minutes, respectively in experiment A, and at 0 minutes in experiment B. The blood gas analysis of the femoral artery was also monitored prior to the CBF recordings.

CBF Recordings

CBF was recorded by a Laser Doppler cerebral blood flow monitoring apparatus (DRT4, Moor Instruments Inc. Wilmington, USA), connected to a personal computer, which was used to collect and analyze the data.

This study was divided into experiment A and experiment B. In experiment A, the animals were divided into four groups of six rats each as follows: (1) sham group: both common carotid arteries were exposed, but the CBF was not blocked; (2) 2 Hz EA without CI group (2HzEA – CI): both common carotid arteries were exposed, but the CBF was not blocked (2 Hz EA applied to both the Zusanli and Sanyinjiao acupoints); (3) 2 Hz EA

with CI group (2HzEA + CI): the CBF of both common carotid arteries was blocked, and 2 Hz EA was applied to both the Zusanli acupoints; and (4) 15 Hz EA with CI group (15HzEA + CI): the methods were identical to the 2HzEA + CI groups, except that 15 Hz EA was applied.

Experiment A was divided into three periods. CBF was recorded for 30 minutes in each period as follows. (1) Baseline (B) period: the baseline CBF was recorded prior to EA stimulation. (2) EA (EA) period: following B period, the acupuncture needles (stainless, 1.27 cm in length) were inserted into the muscle layer (2 mm depth) at both the Zusanli and Sanyinjiao acupoints, and the acupuncture needles were connected to an EA apparatus (Han's Healthronics Likon, Taipei, Taiwan). The electric stimuli were delivered through Zusanli and Sanyinjiao acupoint needles. Zusanli acupoint needles were used as a cathode, while Sanyinjiao acupoint needles as an anode. The intensity of the stimulus was 5 mA during which muscle twitch was slightly visible. (3) Post-EA (P) period: following EA period, the electrical stimulation was stopped and acupuncture needles were removed. In addition, 0.5 ml of whole blood was drawn from the right femoral artery for NO measurement at the end of B, EA and P periods, respectively. Finally, the rats were sacrificed and the brains were removed. Cerebral cortex and thalamus tissue from the right side of the brain were isolated. The brain tissues were suspended and used to measure CGRP.

In experiment B, the animals were divided into two groups of six rats each as follows. (1) 2 Hz EA plus L-NAME (2HzEA + L-NAME) group: the CBF of both common carotid arteries was blocked; 2 Hz EA was applied to both the Zusanli acupoints, and L-NAME (20 mg/kg) was administered. (2) 15 Hz EA plus L-NAME (15HzEA + L-NAME) group: the method was identical to that used in the 2HzEA + L-NAME group, except 15 Hz EA was applied instead of 2 Hz EA.

The experiment was divided into three periods as follows. (1) Baseline (B) period: The baseline CBF was recorded for 20 minutes prior to EA stimulation. (2) EA period: following the B period, the acupuncture needles were inserted into both the Zusanli and Sanyinjiao acupoints, and connected to an electroacupuncture apparatus. The electric stimuli were delivered through Zusanli and Sanyinjiao acupoint needles. Zusanli acupoint needles were used as a cathode, while Sanyinjiao acupoint needles as an anode. The CBF was recorded for 20 minutes. (3) L-NAME period: L-NAME 20 mg/kg was administered via the right femoral vein after 20 minutes EA stimulation; the CBF was then recorded for 40 minutes.

The mean CBF at baseline was used as the denominator (100%) and compared to the mean CBF during the EA period, and the L-NAME period.

Measurement of NO in Blood

NO was generated from the final products of the reaction between nitric oxide and ozone. The levels of NO were calculated by a gas-phase chemiluminescent NO analyzer (NOA^{TM} 280i, Sievers Instrument Inc., USA). NO reacts with oxyhemoglobin and superoxide anion to form nitrate; therefore vanadium (III) chloride in hydrochloric acid was used to convert nitrate to NO. Briefly, 0.5 ml of whole blood was placed into a 1.5 ml tube; and 1.0 ml cold

ethanol (0°C) was added and homogenized with a vibrator for 1 minute. The sample was stood for 30 minutes and centrifuged at 14,000 rpm for 5 minutes. The supernatant was used to determine the NO level. The NO levels were determined by a standard curve of sodium nitrate whose concentration was known.

Measurement of CGRP in Brain Tissue

A rat CGRP ELISA kit (SpiBio, France) was placed into different concentrations of protein suspension as a marker. The levels of CGRP were then determined by a colorimetric method (optical density) and an ELISA reader (Tecan, Sunrise Absorbance Reader, France).

Statistical Analysis

The data are presented as mean ± SD. One-way analysis of variance (ANOVA) followed by the Scheffe's test was used for comparisons between periods. A $p < 0.05$ was considered statistically significant.

Results

Effect of EA on CBF in Rats With and Without CI

In the sham group, the mean CBF was 100 ± 0.0% in the B period, 103.7 ± 5.00% in the EA period and 102.3 ± 7.6% in the P period. No significant difference was noted between them (all $p > 0.05$, Fig. 1).

In the 2HzEA − CI group, the mean CBF was 137.7 ± 12.6% in the EA period which is greater than 100 ± 0.0% in the B period ($p < 0.001$, Fig. 1) and 112.8 ± 13.2% in the P period ($p < 0.01$, Fig. 1).

In the 2HzEA + CI group, the mean CBF was 195.7 ± 53.1% in the EA period, greater than 100 ± 0.0% in the B period ($p < 0.001$, Fig. 1). The mean CBF was 142.3 ± 27.7% in the P period which did not differ significantly from the EA period ($p > 0.05$, Fig. 1). The mean CBF was also not significantly different between the B and P periods ($p > 0.05$, Fig. 1).

In the 15HzEA + CI group, the mean CBF was 206.3 ± 69.9% in the EA period which is greater than 100 ± 0.0% in the B period ($p < 0.01$, Fig. 1). The mean CBF in the EA period did not significantly differ from 153.0 ± 44.1% in the P period ($p > 0.05$, Fig. 1). The mean CBF was also not significantly different between the B and P periods ($p > 0.05$, Fig. 1).

The Time Course of Systolic Blood Pressure in EA-treated Rats With and Without CI

Blood pressure was monitored for a total of 90 minutes. Systolic blood pressure was recorded at the beginning of the experiment at 0 minutes (0), 30 minutes just at the beginning of EA (30), 60 minutes just at the end of EA (60), and 90 minutes just at

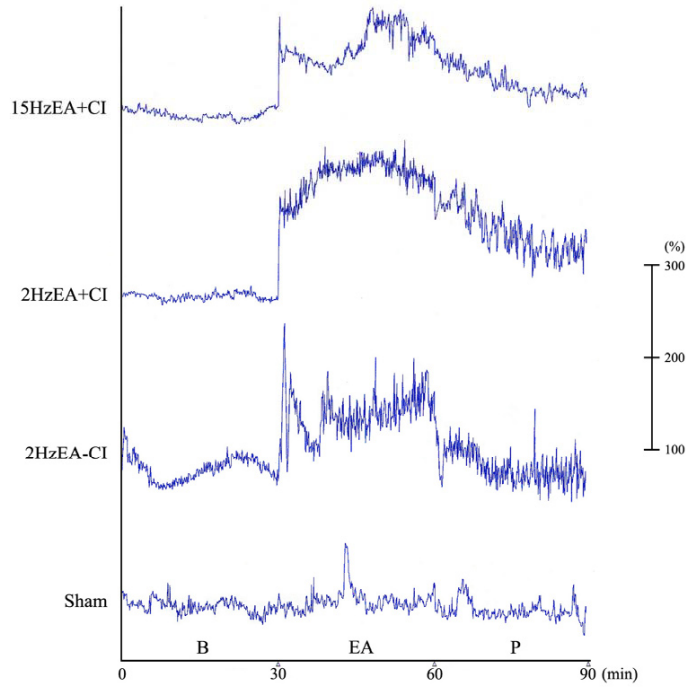

Figure 1. Effect of EA on cerebral blood flow in rats with and without cerebral ischemia. Sham: sham group without EA stimulation or cerebral ischemia; 2HzEA − CI: 2 Hz EA stimulation at both Zusanli acupoints, but no cerebral ischemia; 2HzEA + CI: 2 Hz EA with cerebral ischemia; 15HzEA + CI: 15 Hz EA with cerebral ischemia; B: cerebral blood flow recordings of baseline period; EA: cerebral blood flow recordings of EA period; P: cerebral blood flow recordings of post-EA period.

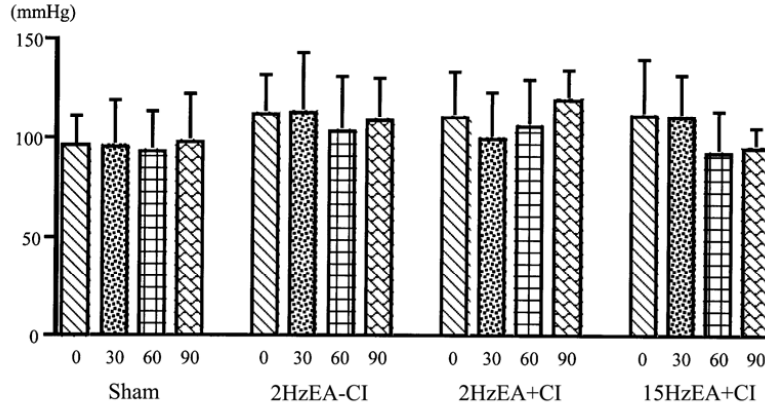

Figure 2. Time course of systolic blood pressure in EA-treated rats with and without cerebral ischemia. The systemic blood pressure was recorded at the beginning of experiment (0), 30 minutes just at the beginning of EA (30), 60 minutes just at the end of EA (60) and 90 minutes just 30 minutes after completion of EA (90) (Fig. 2). The systolic blood pressure did not differ significantly among the sham, 2HzEA − CI, 2HzEA + CI and 15HzEA + CI groups at 0, 30, 60, and 90 minutes.

30 minutes after EA stopped (90), respectively (Fig. 2). There was no significant difference in systolic pressure among the four groups at 0, 30, 60, and 90 (all $p > 0.05$, Fig. 2).

Effect of EA on CGRP in Rats With and Without CI

The CGRP levels in the cerebral cortex were 8.165 ± 2.384 pg/ml in the sham group, 10.775 ± 6.983 pg/ml in the 2HzEA − CI group, 9.977 ± 2.536 pg/ml in the 2HzEA + CI, and 10.555 ± 2.958 pg/ml in the 15HzEA + CI group. There were no significant differences between them (all $p > 0.05$, Fig. 3).

The CGRP levels in the thalamus were 13.387 ± 2.514 pg/ml in the sham, 13.730 ± 5.460 pg/ml in the 2HzEA − CI, 17.482 ± 3.686 pg/ml in the 2HzEA + CI, and 15.742 ± 4.253 pg/ml in the 15HzEA + CI groups. There were also no significant differences between them (all $p > 0.05$, Fig. 3).

The Relationship Between Effect of EA on CBF and NO in Rats With and Without CI

In the sham group, NO levels in the peripheral blood were 19.250 ± 8.581 μM in the B period, 19.800 ± 8.925 μM in the EA period, and 23.167 ± 10.614 μM in the P period. There were no significant differences in NO levels between periods (all $p > 0.05$, Fig. 4).

In the 2HzEA − CI group, NO levels in the peripheral blood were 19.933 ± 5.208 μM in the B period, 23.717 ± 5.049 μM in the EA period, and 24.633 ± 4.030 μM in the P period. There were no significant differences in NO levels between periods (all $p > 0.05$, Fig. 4).

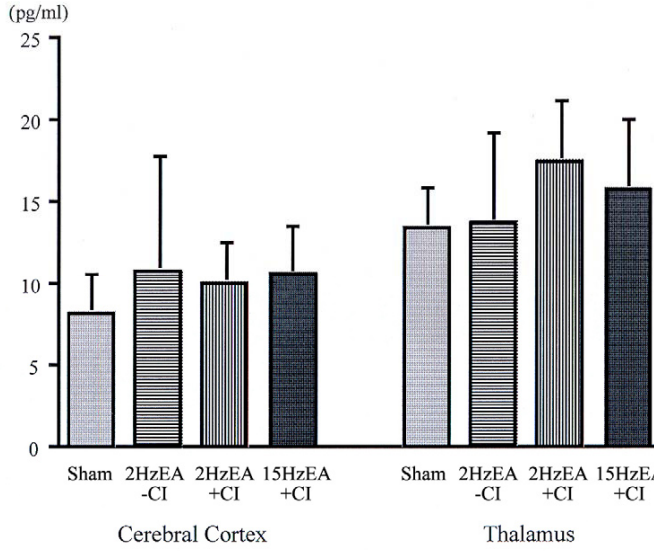

Figure 3. Effect of EA on calcitonin gene-related peptide in rats with and without cerebral ischemia. The levels of CGRP did not differ significantly between the groups. Cerebral cortex: CGRP levels in cerebral cortex; Thalamus: CGRP levels in thalamus.

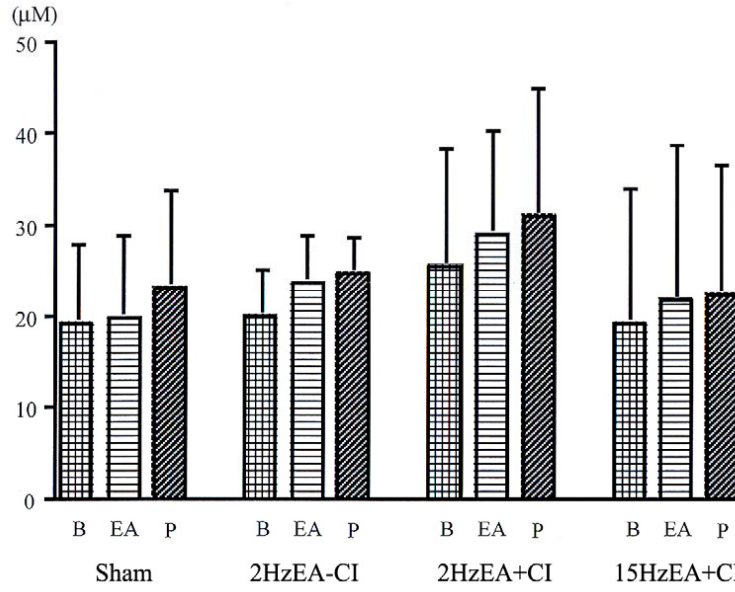

Figure 4. Effect of EA on NO in rats with and without cerebral ischemia. The levels of NO did not differ significantly between the B, EA and P periods in the sham, 2HzEA − CI, 2HzEA + CI and 15HzEA + CI groups.

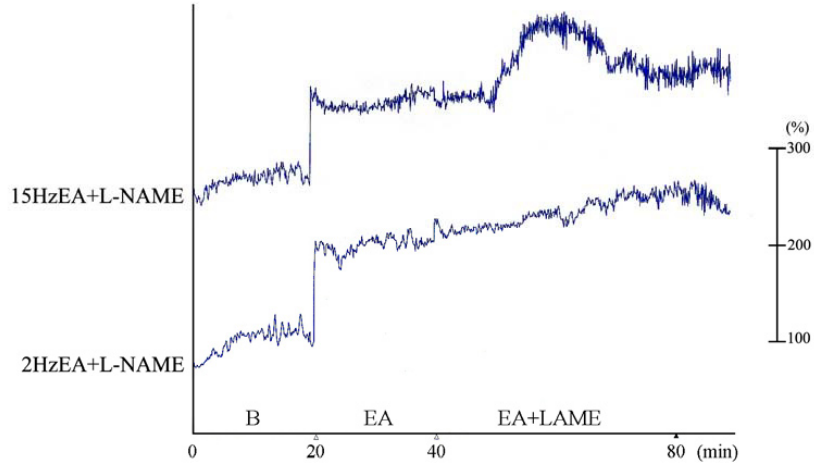

Figure 5. The relationship between EA-induced cerebral blood flow changes and nitric oxide in rats with and without cerebral ischemia. EA induced an increase in cerebral blood flow, but this increase was not altered by L-N (G)-nitro arginine methyl ester (L-NAME). B: cerebral blood flow of baseline period; EA: cerebral blood flow in EA period; EA + L-NAME; cerebral blood flow in the EA + L-NAME period.

In the 2HzEA + CI group, NO levels in the peripheral blood were 25.500 ± 12.850 μM in the B period, 29.033 ± 11.155 μM in the EA period, and 30.983 ± 13.855 μM in the P period. There were no significant differences in NO levels between periods (all $p > 0.05$, Fig. 4).

In the 15HzEA + CI group, NO levels in the peripheral blood were 19.067 ± 14.591 μM in the B period, 21.817 ± 16.807 μM in the EA period, and 22.383 ± 14.020 μM in the P period. There were no significant differences in NO levels between periods. (all $p > 0.05$, Fig. 4).

In the 2HzEA + L-NAME group, the mean CBF was 100 ± 0.0% in the B period, 167.0 ± 31.5% in the EA period, and 165.8 ± 47.5% in the EA + L-NAME period. The mean CBF in both the EA and EA + L-NAME periods was greater than that in the B period (both $p < 0.05$, Fig. 4), whereas the mean CBF was similar in the EA and EA + L-NAME periods ($p > 0.05$, Fig. 5).

In the 15HzEA + L-NAME group, the mean CBF was 100 ± 0.0% in the B period. The mean CBF in the EA (172.8 ± 30.1%) and EA + L-NAME (194.7 ± 29.1%) periods was greater than that in the B period (both $p < 0.001$, Fig. 4), whereas the mean CBF was similar between the EA and EA + L-NAME periods ($p > 0.05$, Fig. 5).

Discussion

Our experiment showed that mean CBF increased after 2 EA and 15 Hz EA were applied to both Zusanli acupoints in rats with CI. The mean CBF also increased during 2 Hz EA stimulation at both Zusanli acupoints in rats without CI. The intensity of stimulus was identical and there is no difference in systolic blood pressure among the groups throughout the course of experiment A. Additionally, the rats were under an anesthetic state without leg movement. Therefore, the 2 or 15 Hz EA increases in CBF in the middle cerebral arterial region were not due to the EA-induced blood pressure changes or leg movement. We found that 2 Hz applied to both Zusanli acupoints induced similar changes in mean CBF between rats with CI and rats without CI; therefore, 15 Hz EA was not applied to rats without CI in the present study. Our results are very similar to those reported in several other studies. For example, acupuncture stimulation increased CBF in the anterior cingulus, insular, superior and inferior frontal gyri areas (Biella *et al*., 2001). EA increased the CBF in the contralateral cerebral hemisphere cortex, thalamus, bilateral cerebellar and ipsilateral basal ganglion (Adams and Assefi, 2001). Acupuncture stimulation at Heku acupoints (Li 4) activated the hypothalamus region of the brain. The hypothalamus has abundant endorphinergic neurons and projections to the raphe nucleus and periaqueductal gray matter of the mid brain, which are regions closely related to the analgesic efficacy of acupuncture (Hsieh *et al*., 2001).

Our results showed that neither 2 nor 15 Hz EA at both Zusanli acupoints induced changes in NO levels in peripheral blood in rats with and without CI. In addition, L-NAME, a non-selective NOS inhibitor, did not change the CBF following stimulation of 2 and 15 Hz EA in rats with CI. Our results also indicated that the levels of CGRP were similar in the cerebral cortex and thalamus region in rats with and without CI in the sham,

2HzEA − CI, 2HzEA + CI, and 15HzEA + CI groups, suggesting that the levels of CGRP are not associated with changes of the CBF in EA-treated rats. However, several studies reported that EA increased blood flow of muscle and skin via the inhibition of sympathetic activities and the release of CGRP (Jansen *et al*., 1989; Johansson *et al*., 1993). EA may produce a dynamic balance between the autonomic nervous system and the release of NO to increase knee joint microcirculation (Loaiza *et al*., 2002), we considered that the mechanism of EA at both Zusanli acupoints induced the increase of the CBF needed further study. Uchida *et al*. (2002) reported that acupuncture increased CBF mainly from the cholinergic system of the nucleus basalis of Meynert in brain, and the afferent pathway of acupuncture impulse was somatic group III and IV afferent nerves (Uchida *et al*., 2002).

In conclusion, 2 and 15 Hz EA at both Zusanli acupoints increases the CBF in the middle cerebral arterial region in rats with and without CI. Whether this effect of EA is related to NO or to CGRP needs to be clarified in a following study.

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