Electroacupuncture improves cardiac function and remodeling by inhibition of sympathoexcitation in chronic heart failure rats

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Ma L, Cui B, Shao Y, Ni B, Zhang W, Luo Y, Zhang S. Electroacupuncture improves cardiac function and remodeling by inhibition of sympathoexcitation in chronic heart failure rats. Am J Physiol Heart Circ Physiol 306: H1464–H1471, 2014. First published February 28, 2014; doi:10.1152/ajpheart.00889.2013.—Chronic heart failure (CHF) is responsible for significant morbidity and mortality worldwide, mainly as a result of neurohumoral activation. Acupuncture has been used to treat a wide range of diseases and conditions. In this study, we investigated the effects of electroacupuncture (EA) on the sympathetic nerve activity, heart function, and remodeling in CHF rats after ligation of the left anterior descending coronary artery. CHF rats were randomly selected to EA and control groups for acute and chronic experiments. In the acute experiment, both the renal sympathetic nerve activity and cardiac sympathetic afferent reflex elicited by epicardial application of capsaicin were recorded. In the chronic experiment, we performed EA for 30 min once a day for 1 wk to test the long-term EA effects on heart function, remodeling, as well as infarct size in CHF rats. The results show EA significantly decreased the renal sympathetic nerve activity effectively, inhibited cardiac sympathetic afferent reflex, and lowered the blood pressure of CHF rats. Treating CHF rats with EA for 1 wk dramatically increased left ventricular ejection fraction and left ventricular fraction shortening, reversed the enlargement of left ventricular end-systolic dimension and left ventricular end-diastolic dimension, and shrank the infarct size. In this experiment, we demonstrated EA attenuates sympathetic overactivity. Additionally, long-term EA improves cardiac function and remodeling and reduces infarct size in CHF rats. EA is a novel and potentially useful therapy for treating CHF.

METHODS

Experimental Animals

Procedures involving rats were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University, and the studies conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). These procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. Male Sprague-Dawley rats weighing between 250 and 300 g were used in the experiments.

Creation of CHF Rats

CHF was induced by the coronary artery ligation technique. All rats were anesthetized with 10% chloral hydrate (0.03 ml/kg ip). Surgery
was carried out with sterile techniques. The left anterior descending coronary artery was ligated with a 6-0 suture near its branch point from the aorta, between the pulmonary artery outflow tract and the left atrium. Mortality was nearly 40% in these experiments, and death occurred primarily during the first day after ligation. The rats were caged in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow and tap water were available ad libitum. The sham-surgery rats were operated the same as the heart failure rats except ligation of their coronary arteries. The terminal experiment was carried out 4 wk after coronary ligation or sham surgery. CHF model rats were all confirmed by specific technician using animal echocardiography (Vevo 2100, Detector MS250, FUJIFILM VisualSonics, Toronto, Canada), which was used to evaluate the heart rate, ejection fraction (EF), fraction shortening (FS), left ventricular (LV) end-diastolic dimension (LVEDD), and LV end-systolic dimension (LVESD) through M-mode recordings.

Terminal Experiments and Measurements

In acute experiments, each rat was anesthetized with urethane (800 mg/kg ip) and α-chloralose (40 mg/kg ip). Supplemental doses of anesthetic were administered at 1/10th of the initial dose per hour. A midline incision in the neck was made where the carotid artery was cannulated for measurement of mean arterial pressure (MAP) and heart rate. The external jugular vein was also cannulated for drug infusion.

RSNA Recording

In the rats, a left flank incision was made and a retroperitoneal dissection was used to expose the renal artery and nerves. The renal sympathetic nerves were identified and dissected free of the surrounding connective tissue. The signal was amplified with a Grass direct-current preamplifier (model P18D; Astro-Med, West Warwick, RI) with the low-frequency cutoff set at 30 Hz and the high-frequency cutoff set at 3 kHz. The amplified and filtered signals were integrated at the time constant of 100 ms. This value was subtracted from all the integrated values of RSNA. The raw nerve activity, integrated nerve activity, MAP, and heart rate were recorded on a PowerLab data acquisition system (model 16S; ADInstruments, Mountain View, CA) and stored on disk until being analyzed.

Evaluation of CSAR

The rats were placed on positive-pressure ventilation. The chest was opened through the fourth intercostal space on the left side. The pericardium was removed to fully expose the LV. This preparation was used for anterior LV epicardial application of a piece of filter paper (3 × 3 mm) containing capsaicin (0.4 μg in 2 μl) (M2028, Sigma-Aldrich, St. Louis, MO) for 1 min to induce CSAR. The filter paper was then removed and the epicardium was rinsed three times with 10 ml of warm normal saline (37°C). The CSAR was evaluated by change of the RSNA and MAP responses to epicardial application of capsaicin.

Selection of Acupoint and Performance of Electroacupuncture

The acupoints of the rats are quite analogous to those in humans. Both neiguan and jianshi acupoint [pericardial meridian (P) 5-6] are located on the median nerve. In the rat, stainless needles were placed at the neiguan and jianshi acupoint located 1.5–2.0 and 2.5–3.0 mm above the wrist between the ligaments of the flexor carpi radialis and the palmaris longus, respectively. The criterion for accurate needle positioning was relied on the observation of a slight repetitive flexion of the paw during stimulation (30). The stimulation was set at low current and low frequency (0.5-ms pulses, 2 Hz and 2 mA) by a Hans-200A electrostimulator (Beijing Sheng Da medical instrument center, Beijing, China) for 30 min throughout the study. To exclude the effect of EA current, for the control EA used in the experiment, Pianli-Wenliu acupoints (large intestine 6–7) in the forelimb were selected because they are near the neiguan-jianshi acupoint yet are located along another meridian (large intestine meridian) (51).

Measurement of Infarct Size

The LVs were cut from apex to base into four transverse slices, which were processed in a routine manner for histological study. Sections (5-μm thick) were cut and stained with Masson’s trichrome stain. ImageJ 1.34 software was used to measure lengths and areas of infarct and LV. In each slice, the infarct ratio was defined as the ratio of the length of the endocardial circumference made up by the infarct area to the entire endocardial circumference. Infarct area in each slice was calculated by multiplying the infarct ratio by the LV area of the whole slice. Infarct ratio of the entire LV was obtained by calculating the ratio of the total infarct area to the sum of LV areas in all slices analyzed (5, 36).

Experiment Protocol

Acute experiments. Acute experiments have been consisted of two cohorts. In the first cohort, three groups were prepared for the acute experiments including CHF rats-EA (n = 6), CHF rats-control EA (n = 6), and sham-operated rats-EA (n = 6). After blood pressure, heart rate, and baseline RSNA were stable for at least 10 min during recording, we performed bilateral EA at neiguan and jianshi acupoint in CHF and sham-operated rat groups at 2 Hz and 2 mA for 30 min. The control EA was performed at the same intensity for 30 min as in the other CHF rat group. We tried to discern whether the EA at the neiguan-jianshi acupoint could reduce the RSNA among CHF rats compared with sham-operated rats and control EA-treated CHF rats. In the second cohort, three groups including CHF rats-EA (n = 6), CHF rats-control EA (n = 6), and sham-operated rats-EA (n = 6) were used to record CSAR. After blood pressure, heart rate, and baseline RSNA were stable for at least 10 min, the CSAR elicited by epicardial application of capsaicin was recorded before and 30 min after EA and control EA. We evaluated whether EA at the neiguan-jianshi acupoint could weaken the CSAR among CHF rats compared with sham-operated rats and control EA-treated CHF rats.

Chronic experiments. The rats were divided into three groups: CHF rats-EA (n = 10), CHF rats-metoprolol (n = 6), CHF rats-control EA (n = 7) as well as CHF rats without EA (n = 6). In the chronic experiment, EA was initiated 4 wk after ligation of left anterior descending coronary artery. All rats were treated under anesthesia when EA stimulation was given. We performed bilateral EA at the neiguan-jianshi acupoint in CHF rats. Meanwhile, control EA were performed in another CHF rat group using the Pianli-Wenliu acupoint. The stimulation was given at 2 Hz and 2 mA for 30 min once per day for a total duration of 1 wk. After 1 wk of stimulation, the cardiac structure and function of rats within all groups was assessed by animal echocardiography (Vevo 2100, Detector MS250, FUJIFILM VisualSonics). For comparison with the effects of EA on heart performance, the effective β1-selective blocker metoprolol (Sigma), was used as a control. The CHF rats orally administered the metoprolol on the dose basis of 60 mg/kg once per day for 7 days by direct gastric lavage (42). After the examination of animal echocardiography, the CHF rats were euthanized for heart excision and the ratio of heart weight-to-body weight and infarct size were measured.

Statistical Analysis

The effects of EA on RSNA are expressed and calculated as the percent change from the baseline. The percent changes of the RSNA induced by cardiac sympathetic afferent nerve stimulation were used as an indicator of the sensitivity of the CSAR. The
improvement of cardiac function and structure in the CHF rats was evaluated by the change of animal echocardiography results after 1 wk of EA treatment. Two-way ANOVA associated with the Newman-Keul’s test for post hoc analysis was used when multiple comparisons were made. Other comparisons were assessed by Student’s paired t-test. All statistical analysis were done using computer software (SPSS; Chicago, IL). All data were expressed as means ± SE. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Effects of Coronary Ligation on Baseline Hemodynamics and Heart Weight**

The measurements of cardiac function and MAP in sham-operated ($n = 17$) and CHF ($n = 29$) rats are shown in Table 1. It is readily observed that the CHF rats have a significant decrease in EF and FS as well as MAP compared with those measures in sham-operated groups ($P < 0.05$). Additionally, LVESD and LVEDD were significantly enlarged in CHF rats compared with sham-operated rats ($P < 0.05$). Table 1 also demonstrates that heart weight and the ratio of heart weight to body weight were significantly greater in CHF group rats than in sham-operated group rats ($P < 0.05$). Overall, these data indicate the presence of myocardial damage and suggests a decreased contractile function and cardiac remodeling in CHF rats.

**Effects of EA on RSNA and MAP**

A representative recording of effects of EA on RSNA and MAP is shown in Fig. 1. As can be seen, EA significantly lowered the MAP and RSNA in CHF rats compared with

![Table 1. Effects of coronary ligation on baseline hemodynamics and heart weight in CHF and sham-operated rats](image)
As shown in Fig. 2, EA significantly reduced RSNA and MAP in CHF rats. In contrast, control EA had no significant effects on RSNA and MAP in CHF rats ($P < 0.05$). However, the sham-operated rat group that received EA could show a decrease in RSNA and MAP as well. Importantly, EA reduced RSNA and MAP in CHF rats more significantly than in sham-operated rats ($P < 0.05$).

**Effects of EA on CSAR Elicited by Epicardial Application of Capsaicin**

The representative recording of effects of EA on CSAR before and after EA is shown in Fig. 3, which demonstrates that there is significant inhibition of CSAR in CHF rats after EA. Figure 4 demonstrates that RSNA and MAP responses to epicardial application of capsaicin are significantly enhanced in CHF rats than in sham-operated rats before EA ($P < 0.05$) (Fig. 4, A and B). After the treatment of EA, the increase in RSNA and MAP induced by capsaicin among CHF rats were significantly inhibited compared with those measures among sham-operated rats and control EA-treated CHF rats ($P < 0.05$). The control EA had no significant effect on RSNA and MAP by capsaicin in CHF rats.

**Effects of Long-Term EA on the Cardiac Function, Remodeling, and Infarct Size**

Figure 5 demonstrates that 1 wk of EA treatment could significantly improve the heart function and structure in CHF rats. One-week-long EA dramatically increases the EF (Fig. 5A) and FS (Fig. 5B) and reverses the enlargement of LVEDD (Fig. 5C) and LVESD (Fig. 5D) in CHF rats compared with control EA-treated CHF rats and CHF rats without treatment ($P < 0.05$). The effects of EA stimulation are comparable with the effects of $\beta_1$-selective blocker metoprolol on heart function and structure. Additionally, Fig. 6 shows the EA could as well shrink the infarct size in CHF rats compared with non-EA-treated CHF rats ($P < 0.05$), which is consistent with effects of metoprolol, but the effects of EA are weaker than that of metoprolol in reducing the infarct size ($P < 0.05$). However, there was no significant difference in ratio of heart weight to body weight among EA-treated CHF rats and control EA-treated CHF rats and CHF rats without treatment.

Fig. 2. Effects of 30-min EA on percentage of RSNA change (A) and MAP change (B) in sham-EA, CHF-EA, and CHF-control EA rats are shown. *$P < 0.05$, compared with CHF-control EA; †$P < 0.05$, compared with sham-EA.

Fig. 3. Representative recording of RSNA and MAP change induced by epicardial application of capsaicin before and after EA in CHF rats. A: cardiac sympathetic afferent reflex (CSAR) before EA in CHF. B: CSAR after EA in CHF. At arrows, the capsaicin was applied.
DISCUSSION

CHF originates from an impairment of the systolic and the diastolic functions of the heart. The main causes of CHF today are ischemic heart disease and hypertension as well as cardiomyopathy (11). Despite optimized heart failure medication, CHF continues to cause significant morbidity and mortality rates worldwide, demonstrated by a 5-yr survival rate of 25% in men and 38% in women (17), making it highly desirable to discover another effective way to treat CHF. This article is the first animal study to directly examine the effects of EA on sympathetic activity and progression of CHF after left anterior descending coronary artery ligation in CHF rats. We found...
the prognosis in CHF (6). Therefore, reduction of sympathetic activity have been highly relevant to both disease severity and (44). Increased sympathetic drive and reduced parasympathetic adaptive process is a key factor in the pathophysiology of CHF on cardiac function and remodeling, and, therefore, the mal-ever, excessively sustained activation has a devastating impact heart compensate for deteriorating pumping function. How-

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present study was to test the hypothesis that EA can be
beneficial to modulate sympathetic activity in the CHF state and improve the function and structure of the debilitating CHF as well as reducing the infarct size, which could be comparable with effects of the β-blockade drugs like metoprolol. We provide the evidence that EA could be used in an alternative way, at least in a complementary way to treat CHF.

The mechanisms underlying CHF are partially understood. Mounting evidence indicates that neurohumoral activation, especially autonomic dysfunction including sympathetic overactivity, contributes to the progression of CHF (23, 33, 44, 47). In fact, sympathetic activation is even present early in the course of LV dysfunction (2, 14). In an animal model of early LV dysfunction without overt heart failure, among which it has shown that sympathetically influenced, low-frequency heart rate variability was significantly increased, and parasympa-
therapeutically mediated, high-frequency variability was signifi-
cantly decreased (10). Initially, the increased sympathetic nervous system activity during the early phase of CHF helps the heart compensate for deteriorating pumping function. How-
ever, excessively sustained activation has a devastating impact on cardiac function and remodeling, and, therefore, the mal-adaptive process is a key factor in the pathophysiology of CHF (44). Increased sympathetic drive and reduced parasympathetic activity have been highly relevant to both disease severity and the prognosis in CHF (6). Therefore, reduction of sympathetic overactivity has become the cornerstone of CHF treatment. Most recent strategies have focused on preventing organs from

EA reduces the RSNA and inhibit CSAR, which is sensitized in the CHF state. Additionally, EA plays a potentially useful role in improving cardiac function and remodeling and reducing infarct size. The main objective of the present study was to test the hypothesis that EA can be beneficial to modulate sympathetic activity in the CHF state and improve the function and structure of the debilitating CHF as well as reducing the infarct size, which could be comparable with effects of the β-blockade drugs like metoprolol. We provide the evidence that EA could be used in an alternative way, at least in a complementary way to treat CHF.

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high sympathetic drive by reducing the sympathetic overactivity such as β-adrenergic blockers (9).

Sympathetic overactivity may also directly and indirectly exert adverse effects on the structure and function of the heart. Reportedly, increased norepinephrine release is relevant to the development of LV hypertrophy and exerts toxical effects on cardiac fibroblasts and myocytes (39, 41). In addition, high-level norepinephrine acts through the β-adrenergic pathway to stimulate apoptosis in adult rat ventricular myocytes in vitro (7) and apoptosis plays an important role in the LV remodeling progression process (35). Clinical and basic studies have demonstrated that sympathoexcitation was relevant to blunted arterial baroreceptor and cardiopulmonary receptor reflexes as well as enhanced cardiac afferent sympathetic reflex, which is a sympathoexcitatory reflex (46, 47). In the brain stem, rostral ventrolateral medulla (rVLM) is well known as the regulation center of sympathetic activity (8). The central abnormalities of regulation for sympathetic nervous system overactivity in CHF have been reported to play a significant role (21). It is well established that brain renin angiotensin system, oxidative stress, and the dysfunction of nitrogen oxide production in the rVLM contribute to the sympathoexcitation and enhanced cardiac afferent sympathetic reflex in CHF (16, 45, 54). Therefore, rVLM is a potentially powerful therapeutic target to suppress the sympathetic overactivation. With the current various therapeutic approaches using β-adrenergic blockers, the prognosis is still not good enough, and it is necessary to avoid the side effects of medication. From the available data, conservative pharmacological therapy is likely to be insufficient.

Acupuncture as a therapeutic intervention is widely accepted and practiced in the United States (1, 33a). Acupuncture can effectively treat postoperative and chemotherapy nausea and vomiting as well as postoperative pain. Acupuncture can also be useful as an effective method to treat other situations, such as addiction, headache, tennis elbow, myofascial pain, osteo-
arthritis, low back pain, and asthma. The neiguan-jianshi acu-
point is most effective point to treat cardiovascular diseases (24, 27, 52). In previous studies, EA at the neiguan-jianshi could inhibit cardiovascular sympathoexcitatory pressor reflex responses triggered by chemical, mechanical, and electrical stimulation in rats (3, 28, 30, 51). For example, stimulation of the median nerve to mimic EA at the neiguan acupoint diminishes regional myocardial ischemia triggered by a sympathetically mediated increase in cardiac oxygen demand in cats with the partial coronary artery occluded (28). However, the exact effects of EA on CHF are still elusive, due to ambiguous outcomes reported by different clinical studies. Therefore, in this article, we tried to examine whether EA could also attenuate the RSNA and CSAR and improve cardiac function and remodeling in CHF rats. The present study confirms our hypothesis that EA improves cardiac function and cardiac remodeling and reduces infarct size by inhibition of sympathetic activity in CHF rats, but the exact mechanisms to explain these changes remain unknown. In previous studies, it has been demonstrated that group-III and -IV afferent pathways in the median nerves were activated during EA, which provide input to a number of regions in the hypothalamus, midbrain, and brain stem that are associated with cardiovascular regulated sympathetic activity (28, 29, 32, 43). Specifically, resection of the median nerve eliminated the therapeutic effect of EA at the neiguan-jiansh acupoint (53). From previous available studies,
EA at neiguan-jianshi could activate μ- and δ-opioid receptors to inhibit sympathetic activity, specifically receptors located in the rVLM (4, 29, 53). Additionally, EA can induce proenkephalin mRNA expression in rVLM, which accounts for reductions in sympathetic outflow (25). EA could result in modulation of the neuronal nitric oxide synthase activity in the hypothalamus to regulate sympathetic outflow in spontaneously hypertensive rats (19). EA could also modulate cardiac autonomic imbalance by increasing the cardiac vagal component of heart rate variability (15, 22). There are studies reporting that vagal nerve stimulation markedly improved the long-term survival of CHF rats through the prevention of pumping failure and cardiac remodeling (26, 50). These effects could explain our finding that the cardiac function and remodeling as well as infarct size reduction in CHF rats could be improved by long-term EA stimulation via the possibility of enhanced vagal drive and inhibited sympathetic activity.

**Clinical Implication and Study Limitations**

This present study shows for the first time that EA attenuates RSNA and CSAR in CHF rats and suggests that EA improves cardiac function and remodeling as well as reducing infarct size to prevent cardiac progression in CHF rats, which may provide evidence that EA could be a useful alternative treatment method to heart failure. In addition to CHF, the sympathetic activity reduction also plays a pivotal role in the treatment of other diseases, such as diabetes and chronic renal failure (40), so it is conceivable that EA is a potentially promising and valuable way to manage the category of these disorders. In this study, the usefulness of EA as a complementary or alternative therapy in animals has herein been established, but the relevant mechanism has not been well addressed. Therefore, more large-scale, long-term trials of research on its clinical efficacy as well as the underlying mechanisms of its effects need to be further performed.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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