Cardioprotection of electroacupuncture against myocardial ischemia-reperfusion injury by modulation of cardiac norepinephrine release

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Zhou W, Ko Y, Benharash P, Yamakawa K, Patel S, Ajijola OA, Mahajan A. Cardioprotection of electroacupuncture against myocardial ischemia-reperfusion injury by modulation of cardiac norepinephrine release. Am J Physiol Heart Circ Physiol 302: H1818–H1825, 2012. First published February 24, 2012; doi:10.1152/ajpheart.00030.2012.—Augmentation of cardiac sympathetic tone during myocardial ischemia has been shown to increase myocardial O2 demand and infarct size as well as induce arrhythmias. We have previously demonstrated that electroacupuncture (EA) inhibits the visceral sympathoexcitatory cardiovascular reflex. The purpose of this study was to determine the effects of EA on left ventricular (LV) function, O2 demand, infarct size, arrhythmogenesis, and in vivo cardiac norepinephrine (NE) release in a myocardial ischemia-reperfusion model. Anesthetized rabbits (n = 36) underwent 30 min of left anterior descending coronary artery occlusion followed by 90 min of reperfusion. We evaluated myocardial O2 demand, infarct size, ventricular arrhythmias, and myocardial NE release using microdialysis under the following experimental conditions: 1) untreated, 2) EA at P5–6 acupoints, 3) sham acupuncture, 4) EA with pretreatment with naloxone (a nonselective opioid receptor antagonist), 5) EA with pretreatment with chelerythrine (a nonselective PKC inhibitor), and 6) EA with pretreatment with both naloxone and chelerythrine. Compared with the untreated and sham acupuncture groups, EA resulted in decreased O2 demand, myocardial NE concentration, and infarct size. Furthermore, the degree of ST segment elevation and severity of LV dysfunction and ventricular arrhythmias were all significantly decreased (P < 0.05). The cardioprotective effects of EA were partially blocked by pretreatment with both naloxone and chelerythrine. These results suggest that the cardioprotective effects of EA against myocardial ischemia-reperfusion are mediated through inhibition of the cardiac sympathetic nervous system as well as opioid and PKC-dependent pathways.

interstitial norepinephrine; microdialysis; opioids; protein kinase C; sympathetic activation

Augmentation of cardiac sympathetic tone during myocardial ischemia has been shown to increase myocardial O2 demand, infarct size, and the occurrence of arrhythmias in animal models (15). Such effects can lead to a further degradation of myocardial performance and worsen the outcomes of a heart attack. Additionally, both in vitro and in vivo studies have indicated that acute myocardial ischemia causes a focal and marked increase in myocardial norepinephrine (NE) in ischemic regions, which may be involved in the pathophysiology of ischemia-induced complications (6, 10, 33). Clinical evidence indicates that acupuncture may have therapeutic effects on coronary heart disease, arrhythmias, angina pectoris, and myocardial infarction (3, 5, 29). The beneficial effects of acupuncture are partially mediated by sympathetic inhibition (19, 24).

In the present study, the Jiashni-Neiguan acupoints (P5–6) were selected because it has been shown that stimulation of these acupoints affects the cardiovascular system. P5–6 acupoints located over the median nerve are commonly used in traditional Chinese clinical medicine to treat coronary heart disease and hypertension (3–5, 8, 9, 29). We have previously demonstrated that low-frequency and low-current electroacupuncture (EA) at P5–6 significantly inhibits the cardiovascular sympathoexcitatory reflex responses to gastric distension in rats (45–47) as well as to chemical stimulation of the gallbladder (40, 44), suggesting that EA potentially can reduce postprandial angina and myocardial ischemia.

Studies in anesthetized cats with partial coronary occlusion have demonstrated that the myocardial ischemia worsened by sympathetic surge can be mitigated by EA at P5–6. Intravenous injection of naloxone or microinjection of naloxone in the rostral ventrolateral medulla reverses the beneficial effects of EA, suggesting the involvement of opioid-related mechanisms in the brain stem (7).

Myocardial ischemia is associated with a marked increase of NE in ischemic tissue (1, 18, 37, 38). Microdialysis allows the in vivo measurement of NE concentrations in myocardial interstitial fluid. In vivo measurement of NE has been used as a surrogate marker of sympathetic nerve activity and has been examined in myocardial ischemia-reperfusion (MIR) (2). In the present study, we investigated the effect of EA on concentrations of interstitial NE during MIR. Based on previous studies demonstrating the role of opioids (25, 36) and PKC (11, 25) in cardioprotection against ischemia-reperfusion injury, we also sought to determine whether PKC and opioids play a role in EA-induced cardioprotection.

The purpose of this study was to determine the effects of EA on LV function, myocardial O2 demand, arrhythmogenesis, infarct size, and in vivo cardiac NE release. We hypothesized that EA exerts cardioprotective effects against MIR injury by the modulation of interstitial NE through an opioid-PKC dependent pathway.

METHODS

Animal model of myocardial ischemia and reperfusion. This animal study was approved by the Chancellor’s Animal Research Committee of the University of California (Los Angeles, CA), and animals were treated in compliance with National Institutes of Health guidelines on the care and use of laboratory animals. New Zealand White rabbits weighing 2.5–4 kg were anesthetized intramuscularly with ketamine...
(15 mg/kg) and xylazine (2 mg/kg) and mechanically ventilated. Complete anesthesia was maintained by isoflurane (1–2%). The ECG was monitored from limb leads. One femoral artery and one jugular vein were cannulated for blood pressure measurement and drug infusion. Animals underwent a median sternotomy to expose the heart. After a recovery of 30 min after the surgical exposure, the left anterior descending coronary artery (LAD) was occluded with a 4.0 silk suture for 30 min followed by 90 min of reperfusion. Successful occlusion was confirmed by the development of a cyanotic anterior ventricular wall, elevated ST segments, and peaked T-waves on the ECG.

The experimental protocols are shown in Fig. 1. The following six groups of rabbits (n = 6 rabbits/group) were studied: 1) no EA before or after ischemia-reperfusion (untreated group); 2) Neiguan-Jianshi (P5–6) acupuncture were electrically stimulated at 2 Hz for 30 min immediately after the onset of LAD occlusion (EA at P5–6 group); 3) acupuncture needles were inserted to P5–6 without either manual or electrical stimulation, which can serve as a strong control (45) (sham acupuncture group); 4) EA at P5–6 at 2 Hz for 30 min immediately after the onset of LAD occlusion with pretreatment of naloxone (3 mg/kg), a nonselective opioid receptor blocker (EANal group); 5) EA at P5–6 with pretreatment of chelerythrine (5 mg/kg), a non-selective PKC inhibitor (EAChe group); and 6) EA at P5–6 with pretreatment with naloxone (3 mg/kg) and chelerythrine (5 mg/kg) (EANal+Che group).

Pressure-volume measurements by conductance catheter. A Millar conductance catheter was used for the continuous measurement of LV pressure and volume. A 3-Fr conductance pressure-volume catheter (Millar Instruments, Houston TX) was placed in the LV apex and connected to a conductance processor (MPVS Ultra, Millar Instruments). Proper electrode position was confirmed by the examination of segmental volume signals. Hemodynamic indexes were obtained from steady-state pressure-volume loops during sinus rhythm. Systolic LV function was assessed by the maximum rate of pressure change (\(dP/dt_{\text{max}}\)), and diastolic LV function was assessed by the minimal rate of LV pressure change (\(dP/dt_{\text{min}}\)) and isovolumetric relaxation time constant (\(\tau\)). The rate-pressure product (RPP), an index of myocardial \(O_2\) demand, was calculated as systolic blood pressure \(\times\) heart rate/1000 (17). LV work efficiency was calculated as stroke work/RPP.

ECG ST segment evaluation. ECGs were recorded continuously before and during MIR in all the animals with the use of the GE Prucka Cardiolab Systems (GE Healthcare). ST segment elevations of >100 \(\mu\)V from baseline were considered as a significant marker of ischemia. The quantitative ST elevation voltages were statistically analyzed.

Arrhythmia scoring system. Atrial and ventricular arrhythmias were monitored for 20 min after the onset of reperfusion. Their incidence and severity were evaluated with a modified arrhythmia scoring system (42) as follows: 0 = no arrhythmia, 1 = atrial arrhythmias or \(<10\) premature ventricular contractions (PVCs), 2 = \(>10\) PVCs, 3 = ventricular tachycardia (1–2 episodes), and 4 = ventricular tachycardia (>3 episodes) or ventricular fibrillation.

Determination of the ischemic risk zone and infarct size. Immediately after euthanasia, the coronary artery was reoccluded, and the area at risk was determined by negative staining. Evans blue (0.25%) was administered via the jugular vein to stain the nonoccluded area of the LV (11). The heart was excised, and the right ventricle and connective tissue were removed, leaving the intact LV. The heart was then frozen and cut into 5-mm slices. Slices were incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC) buffer at pH 7.4 for 15 min at 37°C and then immersed in 10% formalin. The infarct area (TTC negative) and area at risk (TTC stained) were measured by gravimetric analysis. Infarct severity was calculated as a percentage of the infarct size/area at risk (11).

Microdialysis and NE analysis. Myocardial interstitial fluid was sampled using a microdialysis probe (CMA/20) connected to polyethylene tubes. The dialysis probe was perfused with lactated Ringer’s solution at 2.0 \(\mu\)l/min with a CMA/100 microinfusion pump (CMA Microdialysis, Holliston, MA). Dialysate volumes of 20 \(\mu\)l (sampling time: 10 min) were collected in microvials containing 20 \(\mu\)l of a solution of 2% EDTA in 0.08 N acetic acid. Dialysate sampling started immediately after probe implantation. Experimental interventions were not started for 90 min, a period that has been previously

![Fig. 1. Timeline of the experimental protocol. The timing of the interventions is indicated at the bottom: EA, electroacupuncture; Nal, naloxone; Che, chelerythrine. The effects of opioids and PKC on EA-mediated actions were assessed by the intravenous bolus injection of naloxone or chelerythrine alone and the combination of both 10 min before the 30-min ischemia.](image-url)
Table 1. Hemodynamic variables during the course of the experiment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30-min Ischemia</th>
<th>90-min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>EA group</td>
<td>Control group</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>172.6 ± 18.5</td>
<td>174.1 ± 21.9</td>
<td>178.1 ± 21.0</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>89.4 ± 5.2</td>
<td>90.3 ± 5.1</td>
<td>77.2 ± 4.7*</td>
</tr>
<tr>
<td>Rate-pressure product, beats·min⁻¹·mmHg</td>
<td>15.4 ± 2.1</td>
<td>15.7 ± 1.9</td>
<td>13.8 ± 2.3</td>
</tr>
<tr>
<td>End-systolic pressure, mmHg</td>
<td>87.4 ± 6.5</td>
<td>88.7 ± 5.0</td>
<td>75.9 ± 6.7*</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>3.4 ± 0.8</td>
<td>3.9 ± 0.6</td>
<td>6.5 ± 1.1*</td>
</tr>
<tr>
<td>End-systolic volume, ml</td>
<td>2.5 ± 1.1</td>
<td>2.2 ± 0.5</td>
<td>3.7 ± 1.2*</td>
</tr>
<tr>
<td>End-diastolic volume, ml</td>
<td>4.8 ± 1.2</td>
<td>4.6 ± 0.4</td>
<td>5.6 ± 1.0*</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>2.5 ± 0.3</td>
<td>2.4 ± 0.5</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>Stroke work, mmHg·ml</td>
<td>218.2 ± 17.8</td>
<td>212.4 ± 18.7</td>
<td>164.9 ± 16.4*</td>
</tr>
<tr>
<td>dP/dt max, mmHg</td>
<td>3,054.4 ± 325.5</td>
<td>2,948.5 ± 346.8</td>
<td>1,995.7 ± 337.8*</td>
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<td>dP/dt min, mmHg</td>
<td>−315.06 ± 340.9</td>
<td>−326.23 ± 353.5</td>
<td>−2346.7 ± 339.1*</td>
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<tr>
<td>Isovolumetric relaxation time constant, ms</td>
<td>20.4 ± 3.5</td>
<td>20.7 ± 3.6</td>
<td>27.2 ± 3.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD. EA, electroacupuncture; dP/dt max, maximum rate of pressure change; dP/dt min, minimum rate of pressure change. *P < 0.05 vs. baseline; †P < 0.05 vs. the control group.
DISCUSSION

In this study, we demonstrated that 1) EA at P5–6 significantly reduced the deleterious effects of MIR by attenuating the increase in interstitial NE, which is released in response to myocardial ischemia, and 2) the cardioprotective effects of EA were reduced by naloxone or chelerythrine alone and completely blocked by pretreatment with naloxone and chelerythrine together. Our data suggest that the cardioprotective effects of EA against MIR are mediated through inhibition of cardiac sympathetic nerve activity via opioid- and PKC-dependent pathways.

Previous studies (3, 5, 29) have suggested that acupuncture might reduce angina in patients with ischemic heart disease. These clinical observations have been confirmed experimentally in cats with demand-induced ischemia (7, 20). These studies have shown that acupuncture reduces myocardial ischemia by reducing O2 demand, mainly by inhibiting the reflex elevations in blood pressure during visceral afferent stimulation. Other studies, which used electrical stimulation of the hypothalamus or the midbrain to induce the fight-or-flight response, have shown that EA at P6 and ST36 reduced sympathoexcitatory responses and the frequency of ventricular

Fig. 2. Effects of EA on the left ventricular (LV) work efficiency. A: representative tracings of heart rate [in beats/min (bpm)] at baseline, during myocardial ischemia (30 min), and after reperfusion (90 min). B: graphical representation of the effect of EA on LV work efficiency at baseline, ischemia, and reperfusion. *P < 0.05 vs. baseline; †P < 0.05, EA group vs. control group.

Fig. 3. EA decreased the electrocardiographic ST segment during myocardial ischemia and reperfusion. A: representative ECG tracings from rabbits with and without EA at baseline, during ischemia, and after reperfusion. B: ST segment height (in μV) was reduced by EA during ischemia and reperfusion. *P < 0.05 vs. baseline; †P < 0.05, EA group vs. control group.
extrasystoles (43). Lujan et al. (23) reported that EA lowered the incidence of ischemia-reperfusion-induced ventricular tachycardia and reduced myocardial O2 demand in a conscious rat model of myocardial ischemia. The authors demonstrated that EA reduced heart rate and blood pressure and decreased the incidence of ventricular tachycardia/fibrillation during reperfusion, speculating that acupuncture reduced sympathetic outflow. Lowering the myocardial O2 demand might reduce the supply-demand imbalance and thereby lessen the extent of ischemia during vascular occlusion.

Consistent with previous studies, we have confirmed that EA stimulation reduced MIR-induced increases in infarct size, arrhythmias, and myocardial O2 demand. The observation that EA attenuates the increase in interstitial NE in response to MIR has not been reported previously. Interstitial NE levels have been used and validated as a surrogate for cardiac sympathetic nerve activity (2, 32). The quantitative measurement of myocardial NE levels is of great value in understanding the pathophysiological complications induced by acute ischemia. A previous study (1) has demonstrated that myocardial interstitial NE concentrations increase 10-fold in ischemic territories while remaining unchanged in nonischemic regions. In the present study, the increase of interstitial NE associated with myocardial ischemia was markedly suppressed by EA, suggesting that EA reduces the activity of the sympathetic nervous system in the heart. Of note, EA at P5–6 significantly decreased the heart rate during MIR. The decrease in heart rate during EA may be due to inhibition of sympathetic nerve activity or enhancement of vagal cardiac nerve activity, which has been shown to exert cardioprotection against MIR injury (26). The interaction between EA and vagal cardiac nerve activity in cardioprotection deserves further study.

Importantly, the attenuation of NE increase and cardioprotection with EA were partially blocked by an intravenous injection of naloxone. This is consistent with a previous finding showing that naloxone reverses the beneficial effect of EA on ischemia-induced wall motion abnormalities (7). Previous studies (12–14, 44) have demonstrated that EA produces opioids in the central nervous system and, in turn, decreases sympathetic outflow during the sympathoexcitatory reflex response. We, therefore, speculate that the cardioprotection conferred by EA may be in part through the suppression of NE release in the ischemic region by inhibition of sympathetic outflow via activation of the opioid system in the central nervous system.

Stimulation of opioid receptors during myocardial infarction has been shown to be cardioprotective in several species (35, 36). By exerting antiadrenergic effects, opioids may play an important role in protecting myocytes from Ca2+/H+ overload or arrhythmias. Previous studies (11, 25) have demonstrated that opioid stimulation has an antiarrhythmic effect during cardiac reperfusion. Additionally, intravenous morphine has been shown to reduce infarct size via a chelerythrine-sensitive...
mechanism in the isolated rabbit heart (25), implicating the role of PKC in cardioprotection through the opioid system (11, 22, 39). Our results show that pretreatment with chelerythrine partially blocked the effects of EA, indicating that PKC also likely mediates the EA-mediated cardioprotection.

PKC activation has been shown to activate ATP-sensitive K⁺ (K_{ATP}) channels in rabbit ventricular myocytes (21). Furthermore, activation of PKC by opioids may induce cardioprotection via stimulation of the K_{ATP} channel. Miura et al. (26a) have demonstrated that a K_{ATP} channel opener drug, nicorandil, attenuates ischemia-induced release of NE by 76% and that its effect was abolished by glibenclamide (a K_{ATP} channel antagonist), suggesting that the opening of K_{ATP} channels is cardioprotective via neuromodulation. It is possible that the activation of K_{ATP} channels on sympathetic nerve terminals (16, 31) exerts myocardial protection by reducing the excessive release of NE due to ischemia (27). Although the role of opioid-PKC interactions in the cardioprotection mediated by EA needs further investigation, we would speculate that the cardioprotective effect of EA is mediated by inhibition of sympathetic outflow as well as PKC signaling pathway via activation of the opioid system (Fig. 7). These data provide valuable insights that will guide our future studies.

There are several limitations to the present study. First, the use of intravenous naloxone does not allow the identification of the anatomic site(s) of opioid inhibition. Therefore, we were not able to determine whether opioid action is mediated though the central nervous system or by direct action on the heart. However, we (12–14, 44) have previously demonstrated that EA exerts sympathoinhibition via the activation of opioid

![Fig. 7. Proposed pathways involved in the cardioprotection of EA against ischemia-reperfusion injury. EA induces the release of endogenous opioids in the central nervous system and, in turn, attenuates the sympathetic outflow to suppress NE release in ischemic regions. EA also activates opioid receptors in the myocardium to exert cardioprotection against myocardial ischemia-reperfusion injury via the PKC pathway. K_{ATP}, ATP-sensitive K⁺.](http://ajpheart.physiology.org/)

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receptors in the midbrain and brain stem. Second, in this study, it was assumed that interstitial NE concentrations reflect regional myocardial interstitial NE levels. It is not possible to estimate the absolute value of myocardial interstitial NE concentration from dialysate NE concentration. To overcome this problem, we studied the relative changes in myocardial interstitial NE levels before and during MIR in ischemic regions.

Cardioprotection as a result of opioid receptor stimulation by EA in the heart has clinical implications. A previous study (41) has demonstrated that treatment with naloxone abolished the adaptation to ischemia observed in patients after repeated balloon inflations during coronary angioplasty and induced greater severity and shorter time to onset of chest pain compared with placebo-treated patients. Excessive accumulation of NE due to myocardial ischemia is deleterious to the ischemic myocardium, leading to an expansion of the infarct and stunned myocardium and a greater incidence of reperfusion arrhythmias (28, 30, 34). Considering this evidence, the cardiotoxicity caused by the exposure of the ischemic myocardium to a high concentration of NE could be attenuated by EA. In clinical practice, EA may be beneficial in any form of cardiac ischemia, including myocardial infarctions, cardiac arrest, and heart operations, where the myocardium is exposed to global or regional ischemia-reperfusion injury.

In conclusion, EA at P5–6 decreased MIR-induced ventricular dysfunction, infarct size, the occurrence of arrhythmias, and elevated interstitial NE levels through opioid- and PKC-dependent mechanisms. Our data suggest that EA produces cardioprotective effects against MIR through an inhibition of the cardiac sympathetic nervous system and may play an important role in the clinical treatment of cardiac ischemia. Future studies are needed to elucidate the detailed mechanisms of such cardioprotective effects and its clinical impact.

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

AUTHOR CONTRIBUTIONS

REFERENCES


