Electroacupuncture decreases the susceptibility to ventricular tachycardia in conscious rats by reducing cardiac metabolic demand

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Lujan HL, Kramer VJ, DiCarlo SE. Electroacupuncture decreases the susceptibility to ventricular tachycardia in conscious rats by reducing cardiac metabolic demand. Am J Physiol Heart Circ Physiol 292: H2550–H2555, 2007. First published January 5, 2007; doi:10.1152/ajpheart.00979.2006.—Reperfusion after a brief period of cardiac ischemia can lead to potentially lethal arrhythmias. Clinical observations and experimental work with animals suggest that acupuncture may have therapeutic effects for individuals with coronary heart disease, certain arrhythmias, and myocardial ischemia. Therefore, we tested the hypothesis that electroacupuncture reduces the susceptibility to ischemia-reperfusion-mediated ventricular tacharyrhythmias. To test this hypothesis, we measured the susceptibility to ventricular tacharyrhythmias produced by 3 min of occlusion and reperfusion of the left main coronary artery in conscious rats under two experimental conditions: 1) control and 2) with electroacupuncture. Acupuncture was simulated by electrically stimulating the median nerves, corresponding to the Jianshi-Neiguan [pericardial meridian (P) 5-6] acupoints. Results document a significantly lower incidence of ventricular tacharyrhythmias with electroacupuncture (2 of 8, 25%) relative to control (14 of 14, 100%) rats. The decreased susceptibility to tacharyrhythmias with electroacupuncture was associated with a reduced cardiac metabolic demand (lower rate-pressure product and ST-segment elevation) during ischemia.

TEMPORARY OCCLUSION of the coronary arteries can lead to potentially lethal arrhythmias (34, 44). The arrhythmias are triggered by the ischemic insult directly or during the reperfusion phase. The mechanisms mediating the arrhythmias during ischemia and reperfusion are related but distinct (4, 28, 35, 44). Although ischemia is a more common trigger of sudden death than is reperfusion, life-threatening reperfusion arrhythmias are observed during relief of coronary spasm, during angio-plasty or thrombolysis, and after cardiac surgery with ischemic arrest (11).

It is clear from clinical studies that acupuncture has therapeutic effects for individuals with hypertension, myocardial ischemia, and certain arrhythmias (1, 3, 23, 38, 40, 46). For example, acupuncture reduced the number of anginal attacks and increased the threshold for angina during exercise (38) as well as increased the maximal rate-pressure product for patients with severe, stable angina during exercise (1). The beneficial effects of electroacupuncture are mediated, in part, by sympathetic inhibition (33, 49). For example, sympathetically mediated increases in cardiac oxygen demand are reduced by stimulation of the median nerve (to mimic acupuncture) in chloralose-anesthetized cats (24).

Based on these clinical and experimental observations, we tested the hypothesis that electroacupuncture reduces the susceptibility to ventricular tachycardia, initiated by occlusion and reperfusion of the left main coronary artery, by reducing cardiac metabolic demand during ischemia (i.e., lowering rate-pressure product and ST-segment elevation). Conscious, chronically instrumented rats were studied to negate the confounding effects of anesthetic agents and surgical trauma.

MATERIALS AND METHODS

Surgical Procedures

Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of Wayne State University. The studies conformed to American Physiological Society guidelines and principles for research involving animals.

Instrumentation. All surgical procedures were performed using aseptic procedures. Female rats ($n = 14$, $261 \pm 15$ g body wt) were anesthetized with pentobarbital sodium (50 mg/kg ip), and supplemental doses (10 mg/kg ip) were administered if the rat regained the blink reflex or responded during the surgical procedures. Subsequently, a telemetry device (Data Sciences International PhysioTel PA-C40: pressure only, $n = 3$; or CS0-PXT: pressure, temperature, and electrocardiogram, $n = 11$) was implanted as previously described (6, 39), and a catheter was placed in the intraperitoneal space for the infusion of fluids. The sensor of the telemetry device, located within the tip of a catheter, was inserted into the abdominal aorta for continuous non-tethered recording of pulsatile arterial blood pressure (PA-C40) or pulsatile arterial blood pressure, temperature, and electrocardiogram (CS0-PXT) via radio telemetry. In the three rats that were instrumented with the pressure-only transmitter, three insulated stainless steel electrocardiogram (ECG) electrodes were sutured subcutaneously on the ventral side of the thorax. Finally, a pair of multistranded, stainless steel, Teflon-coated wires (Medwire, Mount Vernon, NY) were sutured over the Jianshi-Neiguan [pericardial meridian (P) 5-6] acupoints overlying the median nerve (53) in eight animals. Previous studies have documented that the P 5-6 acupoints (overlying the median nerves) on both forelimbs of small animals are analogous to those in humans (16). The correct positioning of the electrodes at the acupoints was confirmed by observing slight repetitive paw twitches during stimulation, indicating stimulation of motor fibers in the median nerve (5, 25, 26). In six animals, Teflon-coated wires were sutured over the Pianli-Wenliu [large intestine (LI) 6-7] acupoints in the forelimb because they are near P 5-6 yet are located along another meridian (large intestine).

The ECG leads (animals with pressure-only transmitter), electroacupuncture leads, and intraperitoneal catheter were exteriorized at the back of the neck. A minimum of 1 wk of recovery was allowed for animals to exceed their presurgical body weight (19). During the recovery period, the rats were handled, weighed daily, and acclimated to the laboratory and investigators (19).

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After recovery, the animals were anesthetized as described above, and the hearts were approached via a left thoracotomy through the fourth intercostal space. A coronary artery occluder, made from 5.0-gauge atraumatic prolene suture (8720H, Ethicon), which passed through a polyethylene-50 guide tubing (Clay Adams), was passed around the left main coronary artery 2 to 3 mm from the origin by inserting the needle into the left ventricular wall under the overhanging left atrial appendage and by bringing it out high on the pulmonary conus (7, 8, 22, 27). The guide tubing with the other end of the occluder was then exteriorized at the back of the neck. The tubing was filled with a mixture of vasoline and mineral oil to prevent a pneumothorax. Again, at least 1 wk was allowed for recovery. During the recovery period, the rats were handled, weighed daily, and acclimated to the laboratory and investigators. Two separate surgeries, separated by at least 1 wk, were performed because the animals recover significantly better than if two major surgeries are conducted during one session.

**Experimental Procedures**

**Susceptibility to ischemic-reperfusion-induced arrhythmias.** Conscious, unrestrained rats were studied in their home cages (~13,350 cm³) for all experiments. Rats were allowed to adapt to the laboratory environment for ~1 h to ensure stable hemodynamic conditions. Subsequently, electroacupuncture was initiated (0.5-ms pulse duration, 2 Hz, at a current intensity sufficient to produce moderate paw twitches) for 30 min. Thirty minutes of low-current, low-frequency (0.5-ms pulses, 2 Hz, 2–5 V, 1–4 mA) electroacupuncture has been used with cats (13) and rats (9).

This stimulation frequency was similar to that used in clinical electroacupuncture (36). After 30 min of stimulation, the left main coronary artery was temporarily occluded for 3 min by use of the prolene suture. The electroacupuncture was continued during the occlusion and for 1 min following release of the occluder. Specifically, acute coronary artery occlusion was performed by pulling up on the suture that was around the left main coronary artery and holding the occlusion for 3 min. Rapid changes in the ECG (ST-segment elevation) and arterial pressure occurred within seconds of pulling on the suture, documenting coronary artery occlusion (8, 27). Upon release, the animals exhibited either sustained ventricular tachycardia (Fig. 1) or normal sinus rhythm. When sustained ventricular tachycardia developed, normal sinus rhythm appeared by gently compressing the thorax. Without thorax compression, the sustained ventricular tachycardia progresses to ventricular fibrillation. Upon reperfusion after a 3-min period of occlusion, susceptible rats exhibited immediate...
ventricular tachycardia, which (without intervention) rapidly deteriorated to ventricular fibrillation with a reduction in arterial pressure. However, in most cases, we intervened immediately before ventricular tachycardia culminated into ventricular fibrillation. Ventricular tachycardia was defined as sustained ventricular rate (absence of P wave, wide bizarre QRS complex) >1,000 beats/min with a reduction in arterial pressure below 40 mmHg. Ventricular fibrillation was defined as a ventricular rhythm without recognizable QRS complex, in which signal morphology changed from cycle to cycle and for which it was impossible to estimate heart rate. In the event when the animal did not resume normal sinus rhythm, cardioversion was achieved (after the rat lost consciousness) with the use of one shock (10 J) of direct current. On an alternate day (at least 1 wk later), the protocol was repeated without the electroacupuncture. The order of the protocols, standard control (without electroacupuncture) and electroacupuncture, was randomized.

Although these protocols included a standard control condition without somatic nerve stimulation or electroacupuncture, the best control for an acupoint is to stimulate another acupoint on another meridian that has been reported to have a different function (32). This has been termed the strong control (32). Therefore, based on an earlier study (54), we chose Pianli-Wenliu (LI 6-7) as strong control acupoints because they are near P 5-6 yet are located along another meridian (large intestine). Six additional rats were studied with electrical stimulation of Pianli-Wenliu (LI 6-7) as strong control acupoints, and the protocols were repeated.

**Determination of ischemic zone.** After the experiment, the rats were euthanized with an overdose of pentobarbital sodium. To determine the size of the ischemic zone, the heart was excised with the occluder intact and perfused via the aorta with 30 ml of 0.9% saline to wash out the blood. Subsequently, the left main coronary artery was occluded by tying the suture. Evans blue dye (100 μl, 0.5%) was perfused via the aorta, allowing the dye to infuse into the nonischemic area of the heart and leaving the ischemic regions unstained. The heart was trimmed, leaving only the right and left ventricles; rinsed to remove the excess blue dye; and weighed. The heart was trimmed again, leaving only the ischemic region. The weight of the ischemic zone was expressed as a percentage of total ventricular weight (7, 8, 10, 27, 47).

To determine whether the occlusion produced a myocardial infarction, the heart was sliced transversally into ~1.0-mm sections and incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TCC, Sigma) at 37°C for 20 min. The heart sections were placed between two glass slides and immersed in 10% formalin overnight to enhance the contrast of the stain. TCC staining differentiates viable tissue by reacting with myocardial dehydrogenase enzymes to form a red-brick stain. Necrotic tissue that has lost its dehydrogenase enzymes does not form a red stain and shows up as pale yellow. This stain has been shown to be a reliable indicator of myocardial infarction (12).

**Data Analysis**

There were no statistically significant differences in responses between the standard control (n = 8) and strong control (n = 6) conditions, so these data were combined and compared with the responses obtained during electroacupuncture. All data were expressed as means ± SE. The percentage of animals sustaining ventricular tachycardia in the control conditions and during electroacupuncture was compared by using a χ² analysis. A two-factor ANOVA with repeated measures on one factor with post hoc Student-Newman-Keuls method was used to compare mean arterial blood pressure and heart rate immediately before the occlusion (preoclusion) and immediately before the release of the occluder (preelease) in the control conditions and during electroacupuncture. In addition, unpaired, one-tailed t-tests were used to compare rate-pressure product and ST-segment elevation in the control conditions and during electroacupuncture. The rate-pressure product, an index of myocardial oxygen demand, was calculated as systolic blood pressure × heart rate/1,000 (18).

**RESULTS**

Figure 2 presents the percentage of rats in the control conditions and during electroacupuncture that experienced ventricular tachycardia upon release of the coronary artery occluder. One-hundred percent (14 of 14) of the rats in the control conditions (8 of 8 for standard control and 6 of 6 for strong control) and 25% (2 of 8) of the rats during electroacupuncture experienced ventricular tachycardia upon release of the coronary artery occluder. The χ² indicated that this difference was statistically significant at the P < 0.05 level.

Figure 3 presents the ST-segment elevation (Fig. 3A) and rate-pressure product (Fig. 3B) immediately before release of the occluder (preelease) in the control conditions (standard control and strong control combined) and during electroacupuncture. The unpaired, one-tailed t-tests revealed a significantly lower ST-segment elevation and rate-pressure product during electroacupuncture.

Table 1 presents resting mean arterial pressure and heart rate immediately before the occlusion (preoclusion) and immediately before the release of the occluder (preelease) in the control conditions (standard control and strong control combined) and during electroacupuncture. Preoclusion mean arterial pressure and heart rate were not different between the control conditions and during electroacupuncture. In contrast, preelease heart rate was significantly higher than preoclusion heart rate in the control conditions only. Importantly, preelease heart rate was significantly higher in the control conditions compared with that during electroacupuncture (462 ± 13 vs. 416 ± 14 beats/min). The ischemic area averaged 50 ± 2% of the total heart weight, and there was no indication of myocardial infarction.

**DISCUSSION**

In this study, we tested the hypothesis that electroacupuncture reduces the susceptibility to ventricular tachyarrhythmias, induced by myocardial ischemia and reperfusion. Specifically,
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we recorded the susceptibility to ventricular tachyarrhythmias induced by myocardial ischemia and reperfusion in conscious rats in the control conditions and during electroacupuncture. Results document a significantly lower incidence of ventricular tachyarrhythmias in rats during electroacupuncture (2 of 8, 25%; Fig. 2) versus the control conditions (14 of 14, 100%). The reduced susceptibility to ventricular tachyarrhythmias was associated with a reduced cardiac metabolic demand during ischemia (lower rate-pressure product and ST-segment elevation, Fig. 3). These data are consistent with several reports. First, acupuncture has been documented to reduce ventricular extrasystoles induced by stimulation of the hypothalamus (48, 55) as well as to enhance the therapeutic effect of antiarrhythmics for frequent ventricular extrasystole (50). In addition, the ventricular fibrillation threshold was increased after electroacupuncture in anesthetized rats (51). Transauricular electroacupuncture also decreased the severity of ventricular arrhythmias during acute ischemia and subsequent reperfusion in rats (37). Although our data suggest a reduced cardiac metabolic demand during ischemia, we cannot rule out the possibility that electroacupuncture increased myocardial oxygen supply. Specifically, DeJongste and his research team (15, 17), using neuromodulation (spinal cord stimulation and transcutaneous electrical nerve stimulation), suggest that neurostimulation improves angina pectoris with a concomitant improvement of myocardial perfusion.

The mechanisms mediating the therapeutic effects of electroacupuncture are related to activation of group III and IV fibers in the median nerves (43). Specifically, resection of the median nerve eliminates the therapeutic effect of electroacupuncture at Jiashii-Neiguan acupoint P 5-6 (56). Stimulation of group III and IV fibers in the median nerves activate μ- and δ-opioid and nociceptin receptors in the rostral ventrolateral medulla, which inhibit sympathetic outflow and reduce cardiovascular metabolic demands (5, 9, 24, 26, 41, 42). The sympathoinhibition (24) may result in reductions in intracellular Ca^{2+} (52). This is suggested because after Ca^{2+} chelation with EDTA, the antiarrhythmic effects of acupuncture were abolished (52).

One of the major manifestations of myocardial reperfusion following a brief period of ischemia is ventricular arrhythmias. These arrhythmias are observed clinically following relief of coronary artery spasm, angioplasty or thrombolysis, and cardiac surgery with ischemia arrest (28). The severity of reperfusion-induced arrhythmias may be related to the heart rate during the ischemic period (4). Specifically, pacing the heart at slower rates led to a frequency-dependent protective effect against reperfusion-induced arrhythmias. Furthermore, autonomic interventions have a heart rate-dependent effect on reperfusion arrhythmias. When heart rate is held constant, neither β-blockade nor vagus nerve stimulation protects against reperfusion arrhythmias (45). In this study, prerelease heart rate was significantly lower during the electroacupuncture, supporting the suggestion that the severity of reperfusion-induced arrhythmias may be related to the heart rate during the ischemic period (4).

Considerable evidence documents that changes in the ST-segment shift are a valid marker of changes in the severity of myocardial ischemia (20). For example, studies in patients document that the ST-segment shift correlates with both metabolic and contractile parameters of myocardial ischemia (14, 21, 30, 31). Therefore, ST-segment changes are widely used as an index of myocardial injury resulting from ischemia in experimental animals (29). Indirect indexes of myocardial oxygen consumption (tension-time index, double product, and triple product) are also used in clinical and experimental studies (2). These indirect indexes are highly correlated with direct measurements of myocardial oxygen consumption. We used ST-segment shifts and double product (rate-pressure

Table 1. Mean arterial pressure and heart rate at preocclusion and prerelease in the control and electroacupuncture conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Electroacupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>106±4</td>
<td>110±4</td>
</tr>
<tr>
<td>Preocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prerelease</td>
<td>113±4</td>
<td>99±8</td>
</tr>
<tr>
<td>Heart rate</td>
<td>380±6</td>
<td>383±12</td>
</tr>
<tr>
<td>Preocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prerelease</td>
<td>462±13*</td>
<td>416±14†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preocclusion, immediately before occlusion of the left main coronary artery; prerelease, immediately before the release of the occluder. *P < 0.05, preocclusion vs. prerelease; †P < 0.05, control vs. electroacupuncture.

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Fig. 3. ST-segment elevation (A) and rate-pressure product (B) immediately before release of the occluder in the control conditions and during electroacupuncture. The unpaired, one-tailed t-tests revealed a significantly lower rate-pressure product and ST-segment elevation during electroacupuncture.
product, Fig. 3) as an index of the severity of myocardial ischemia. It can be seen in Fig. 3 that both indexes were significantly lower during electroacupuncture.

In conclusion, electroacupuncture decreased the susceptibility to ischemia-reperfusion-induced ventricular tachyarrhythmias by decreasing cardiac metabolic demand during ischemia (lower rate-pressure product and ST-segment elevation). It is important to note that the single session of electroacupuncture did not change the resting level of arterial pressure or heart rate but reduced cardiac metabolic demand during ischemia, suggesting a reduction in ischemia-induced sympathetic activity (33).

GRANTS

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REFERENCES

39. Rodenbaugh DW, Collins HL, Nowacek DG, DiCarlo SE. Increased susceptibility to ventricular arrhythmias is associated with changes in


