Electroacupuncture of Neiguan (PC 6) inhibits enhanced voltage-gated sodium currents in ischemic ventricular myocytes

Baoqiang Dong, Chunri Li, Xiaoqing Zhang, Shudong Wang, Zhedong Cheng, Peijing Rong

Abstract

OBJECTIVE: To examine the effect of electroacupuncture (EA) at bilateral Neiguan (PC 6) on voltage-gated Na⁺ currents (INa) and channels (Nav) in ischemic ventricular myocytes.

METHODS: EA serum was prepared from six male adult Sprague-Dawley rats that had received EA at bilateral Neiguan (PC 6). Eighteen ventricular myocytes were prepared from six SD rats using an enzymolysis approach. Myocardial ischemia was mimicked by perfusion of ischemic solution. Whole-cell patch-clamping was used to record three currents evoked from isolated cells. The first current was the control, and recorded in absence of ischemic solution current. The second was the ischemic current, and recorded after perfusion of ischemic solution for 5 min, while the EA current was last, and recorded after perfusion of EA serum for 5 min. Na⁺ kinetic curves were fitted using related formulas.

RESULTS: Compared with those in controls, in the presence of ischemic solution, peak amplitudes of INa significantly increased from −40 mV to +30 mV, and half-maximal inactivation potentials of Nav increased significantly, while half-maximal activation potentials, slope factors and the recovery time from inactivation to activation of Nav were unchanged. Compared with those in the ischemic solution, in the presence of EA serum, peak ischemic current amplitudes significantly reduced from −40 mV to +40 mV, and half-maximal inactivation potentials were restored, while half-maximal activation potentials, slope factors and the recovery time from inactivation to activation of Na⁺ were unchanged.

CONCLUSION: EA at bilateral Neiguan (PC 6) can reduce enhanced INa via restoration of delayed Nav inactivation in ischemic ventricular myocytes.

INTRODUCTION

Neiguan (PC 6), an acu-point of the pericardial meridian, has been widely used for treating cardiovascular disease. Previous studies have found that electroacupuncture (EA) at Neiguan (PC 6) protects myocardial...
cells against ischemia by reducing heart rate, blood pressure, and $O_2$ demand, and in particular the last effect may regulate supply-demand imbalance and thereby lessen the extent of ischemia. These beneficial effects of EA at Neiguan are caused by inhibition of the cardiac sympathetic system.

Generally, cardiac sympathetic nerve excitation causes norepinephrine (NE) release, which in turn binds to $\beta_1$ receptors, triggering sodium $(Na^+)$ influx via voltage-gated $Na^+$ channels $(Na_v)$ and promoting myocardial cell contraction. Thus, $Na^+$ influx is necessary for myocardial contraction. There are various $Na^+$ influx paths including $Na^+_v$, $Na^+-H^+$ and $Na^+-Ca^{2+}$ exchangers, and $Na^+-K^+-Cl^-$ and other co-transporters, although $Na^+$ are the most important. By contrast, the $Na^+$ influx path is almost exclusively dependent on $Na^+$-$K^+$ ATPase. Myocardial ischemia reduces $Na^+$-$K^+$ ATPase activity due to lack of cellular energy, resulting in $Na^+$ overload, which induces $Ca^{2+}$ overload and mitochondrial damage, causing rapid and obvious contractile dysfunction. Attenuation of $Na^+$ overload via inhibition of $Na^+$ influx can preserve mitochondrial energy production in ischemic myocardium and enhance post-ischemic contractile recovery.

In ischemia, cardioprotection mediated by EA at Neiguan $(PC \ 6)$ has been confirmed; however, the effect on voltage-gated $Na^+$ currents $(I_{Na})$ and $Na^+$ kinetics is still not clear. Accordingly, in the present study we examined the electrophysiological mechanism of EA-mediated cardioprotection using the whole-cell patch-clamp technique to investigate the effect of EA at Neiguan $(PC \ 6)$ on $I_{Na}$ and $Na^+$ following rat ventricular myocyte mimicked ischemia.

**MATERIALS AND METHODS**

**EA serum preparation**

Twelve male adult Sprague-Dawley (SD) rats of specific pathogen-free (SPF) grade, [3 months old, weighing $(240 \pm 10)$ g] were purchased from Liaoning Changsheng Biotechnology Company Limited, Benxi, China [SCXK (Liao) 2010-0001]. Animal care procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize suffering. The animal use protocol was reviewed and approved by the Experimental Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine. The EA method at bilateral Neiguan $(PC \ 6)$ has been described previously. After 7 days of acupuncture treatment, six rats were anesthetized with 10% choral hydrate $(10 \ mg/kg)$; hearts were then dissected and mounted on a Langerdorff apparatus (Chengdu, Sichuan, China). Coronary arteries were retrograde-perfused with $Ca^{2+}$-free Tyrode solution for 5 min, and then collagenase $\|$ solution $(0.4 \ mg/mL)$ for 30 min. Next, hearts were dissected, cut into pieces, and transferred into Tyrode solution. The suspension was filtered $(using a 200 \ \mu M \times 200 \ \mu M \ mesh)$ and stored at room temperature in KB solution. Rod-shaped cardiomyocytes were used to form giga-seals with patch pipettes.

Whole-cell patch-clamp recordings were performed at room temperature $(22^\circ \pm 2^\circ C)$ using an EPC10 patch-clamp amplifier (HEKA, Lambrecht, Germany), as described previously. Briefly, signals were filtered at 5 kHz and digitized at a 2-kHz sampling rate. Series resistance was compensated by at least 60%. Leakage and capacitive currents were subtracted on-line using a P/4 subtraction procedure. After giga-seal formation and membrane rupture, cells were stabilized for 3-5 min before starting protocols. Three currents were induced from one single cell sequentially. The first current was the control and recorded in the absence of ischemic solution, the second was recorded after perfusion of ischemic solution for 5 min, and the last after perfusion of EA serum for 5 min.

**Electrophysiological recordings**

Eighteen ventricular cells were isolated from six rat hearts using a collagenase $\|$ method. Briefly, rats were anesthetized with 10% choral hydrate $(10 \ mg/kg)$; hearts were then dissected and mounted on a Langerdorff apparatus (Chengdu, Sichuan, China). Coronary arteries were retrograde-perfused with $Ca^{2+}$-free Tyrode solution for 5 min, and then collagenase $\|$ solution $(0.4 \ mg/mL)$ for 30 min. Next, hearts were dissected, cut into pieces, and transferred into Tyrode solution. The suspension was filtered $(using a 200 \ \mu M \times 200 \ \mu M \ mesh)$ and stored at room temperature in KB solution. Rod-shaped cardiomyocytes were used to form giga-seals with patch pipettes.

Whole-cell patch-clamp recordings were performed at room temperature $(22^\circ \pm 2^\circ C)$ using an EPC10 patch-clamp amplifier (HEKA, Lambrecht, Germany), as described previously. Briefly, signals were filtered at 5 kHz and digitized at a 2-kHz sampling rate. Series resistance was compensated by at least 60%. Leakage and capacitive currents were subtracted on-line using a P/4 subtraction procedure. After giga-seal formation and membrane rupture, cells were stabilized for 3-5 min before starting protocols. Three currents were induced from one single cell sequentially. The first current was the control and recorded in the absence of ischemic solution, the second was recorded after perfusion of ischemic solution for 5 min, and the last after perfusion of EA serum for 5 min.

**Data analysis**

All data were analyzed and fitted using Clampfit 9.0 (vers 9.2.0.09 Axon Instruments, Inc., Sunnyvale, CA, USA) and Origin 8.0 (vers 8.0724, OriginLab Corporation, Northampton, MA, USA). Values were represented as mean ± standard deviation (SD). Statistical
comparisons were performed using Student’s *t*-test. Values of *P*<0.05 were considered significant.

**RESULTS**

**Effect of EA serum on current-voltage (I-V) curves of *I*$_{\text{Na}}$**

*I*$_{\text{Na}}$ was obtained by 20-ms depolarizing pulses from the holding potential of −70 mV to +70 mV at 10-mV steps. Activated inward currents were completely and reversibly blocked by bath application of 0.5 μM TTX (data not shown), indicating that expressed Na$_{\text{v}}$ were TTX-sensitive. In the presence of ischemic solution, peak *I*$_{\text{Na}}$ amplitudes increased significantly from −40 mV to +30 mV (*n*=6; *P*<0.05, *P*<0.01 vs control currents), and in the presence of EA serum, peak *I*$_{\text{Na}}$ amplitudes decreased significantly from −40 mV to +40 mV (*n*=6; *P*<0.05 vs ischemic currents) (Figure 1).

**Effect of EA serum on steady-state activation curves of Na$_{\text{v}}$**

According to I-V curves of *I*$_{\text{Na}}$ currents from −70 mV to +30 mV are converted to conductance (G) using the following formula: G=I/(Vm−Vr), with Vr the reversal potential. Peak conductance values for each test potential were normalized to Gmax and plotted against the test potential to produce voltage-conductance relationship curves that fitted well using the Boltzmann function: G/Gmax=1/[1+exp−(V−Vh)/k], with Vh the voltage at which conductance is half-maximal, and k the slope factor. Our results found that both Vh and k were unaltered by ischemic solution and EA serum (*n*=6, *P*>0.05) (Figure 2, Table 1), suggesting that neither of them altered the steady-state activation kinetics of Na$_{\text{v}}$.

**Effect of EA serum on steady-state inactivation curves of Na$_{\text{v}}$**

Steady-state *I*$_{\text{Na}}$ inactivation was elicited by a 100-ms

---

**Figure 1** Effect of EA on I-V curves of *I*$_{\text{Na}}$

EA: electroacupuncture. Data are presented as mean±SD (*n*=6). *a* *P*<0.05, *b* *P*<0.01, compared with controls; *c* *P*<0.05, compared with ischemic solution.

**Figure 2** Effect of EA on steady-state activation curves of Na$_{\text{v}}$

EA: electroacupuncture. Data are presented as mean±SD (*n*=6).
conditioning prepulse to potentials between -80 mV and +20 mV in 10-mV increments, followed by a 20-ms pulse of -30 mV and holding potential at -70 mV. Peak $I_{Na}$ amplitudes were normalized and plotted against command potentials, with the resulting data well fitted using the Boltzmann function: $I/I_{\text{max}}=1/[1+\exp((V−V_h)/k)]$, with $I/I_{\text{max}}$ being the normalized current, $V_h$ the potential for half-maximal inactivation, and $k$ the slope factor. Our results found that in the presence of ischemic solution, $Na_v$ steady-state inactivation curves shifted significantly in a positive direction, and were restored by EA serum (Figure 3, Table 2).

**Table 1** Effect of EA on $Na_v$ activation parameters ($n=6$, x±s)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Ischemic solution</th>
<th>EA serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_h$ (mV)</td>
<td>-54.0±1.6</td>
<td>-55.8±1.1</td>
<td>-51.9±3.2</td>
</tr>
<tr>
<td>$k$</td>
<td>4.0±1.2</td>
<td>2.8±0.6</td>
<td>5.8±3.3</td>
</tr>
</tbody>
</table>

Notes: EA: electroacupuncture; $V_h$: membrane potential at half-activation; $k$: slope factor.

**Table 2** Effect of EA on $Na_v$ inactivation parameters ($n=6$, x±s)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Ischemic solution</th>
<th>EA serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_h$ (mV)</td>
<td>-43.25±1.58</td>
<td>-32.90±0.37</td>
<td>-36.15±1.32</td>
</tr>
<tr>
<td>$k$</td>
<td>8.83±1.41</td>
<td>6.36±1.32</td>
<td>6.89±1.38</td>
</tr>
</tbody>
</table>

Notes: EA: electroacupuncture; $V_h$: membrane potential at half-activation; $k$: slope factor. *P<0.05, compared with controls.

**Effect of EA on $Na_v$, recovery time course**

Currents were obtained using the following protocol: holding potential at -80 mV followed by a 50-ms conditioning depolarizing pulse (-40 mV) to fully inactivate $Na_v$, and then a 50-ms test pulse (-40 mV) after a series of -90-mV intervals varying from 2-36 ms (in 2-ms increments). Peak $I_{Na}$ values evoked by the conditioning pulse were designated $I_0$, while those evoked by the test pulse were designated $I_1$. The $I_0$ to $I_1$ ratio represents $I_{Na}$ recovery from inactivation. The plot of $I_0$ to $I_1$ vs duration of -80-mV intervals was fitted using a monoexponential function: $I/I_{\text{max}}=A+B\exp(-t/\tau)$, with $\tau$ being the time constant for recovery from inactivation. No significant differences were found (Figure 4, Table 3).

**DISCUSSION**

Myocardial Na$^+$ concentration in ischemic heart increases in an ischemic duration-dependent manner. There are various Na$^+$ influx paths including $Na_v$, Na$^+$-H$^+$ and Na$^+$-Ca$^{2+}$ exchangers, and Na$^+$-K$^+$-Cl$^-$ and other co-transporters, however, Na$^+$ are the most important. By contrast, Na$^+$-K pumps are exclusively responsible for Na$^+$ efflux. Myocardial ischemia leads to rapid reduction of myocardial energy, and Na$^+$ overload eventually occurs. Na$^+$ can enter mitochondria via Na$^+$-Ca$^{2+}$ exchangers and monocarboxylate transporter inhibitor-sensitive Na$^+$ transporters to induce mitochondrial damage directly, leading to contractile failure. Therefore, inhibition of Na$^+$ influx, especially Na$^+$ blockage, may be a promising approach to protect ischemic cardiomyocytes.

In the present study, we found that peak $I_{Na}$ amplitudes significantly increased from -40 mV to +30 mV.
in the presence of ischemic solution, suggesting that mimicking ischemia increased Na\(^+\) influx. After perfusion of EA serum, incremental I\(_{Na}\) peak amplitudes markedly reduced from -40 mV to +40 mV, suggesting that EA at bilateral Neiguan (PC 6) may oppose INa enhancement caused by ischemia, and reduce Na\(^+\) influx. I\(_{Na}\) provides the electrical energy for electrical impulse propagation between myocardiocytes and determines cardiac conduction speed, therefore I\(_{Na}\) inhibition can not only protect ischemic myocardiocytes but also restrict the ischemic area. Indeed, it has been reported that EA at Neiguan (PC 6) restricts the ischemic area and reduces infarct size,\(^{10}\) and potentially the electrophysiological mechanism may be related to EA inhibition of I\(_{Na}\).

Generally, INa intensity is determined by Na\(_v\) kinetics, hence we examined the effect of EA serum on Na\(_v\). We found Na\(_v\) expressed on myocardiocytes were TTX-sensitive, and such channels can complete state transitions between resting, fast activated, and inactivated states in a few milliseconds or less.\(^{22}\) Myocardiocyte depolarization to the threshold potential activates Na\(_v\), and causes a very large but brief I\(_{Na}\). We did not observe a significant shift in Na\(_v\) steady-state activation curves in the presence of either ischemic solution or EA serum, suggesting that neither affected activation kinetics of Na\(_v\). With regards to Na\(_v\) inactivation kinetics, we found that in the presence of ischemic solution, steady-state inactivation curves shifted significantly in a positive direction, indicating that ischemia delayed Na\(_v\) inactivation. It is known that appropriate Na\(_v\) inactivation is essential for effective action potential repolarization;\(^{23}\) however, delayed inactivation can produce large inward Na\(^+\) currents during the cardiac action potential plateau, causing repolarization failure, early post-depolarization, and life-threatening ventricular tachyarrhythmia.\(^{24}\) Accordingly, restoration of abnormal Na\(_v\) inactivation caused by ischemia is cardioprotective. We found that in the presence of EA serum, positively shifted steady-state Na\(_v\) inactivation curves caused by ischemia had shifted in a negative direction, and there was no significant difference in V\(_s\) value compared with controls. This suggests that EA at Neiguan (PC 6) can restore abnormal Na\(_v\) inactivation.

To understand EA-mediated cardioprotection in ischemia, we calculated Na\(_v\) activation intervals based on our Na\(_v\) activation and inactivation results, as the interval between Na\(_v\) activation and inactivation is closely related to I\(_{Na}\) intensity.\(^{25}\) In the absence of ischemic solution, the Na\(_v\) open interval was between -53.97 mV and -43.25 mV and approximately 10.72 mV in width. However, in the presence of ischemic solution, it was between -55.81 mV and -32.90 mV and approximately 22.91 mV in width. These results suggest that the Na\(_v\) activation interval broadened during ischemia, and such a change may contribute to I\(_{Na}\) enhancement in mimicked ischemia. After perfusion of EA serum, the Na\(_v\) activation interval was between -51.90 mV and -36.15 mV with a width of 15.75 mV, less compared with ischemia, suggesting that EA shortened the broadened activation interval caused by ischemic solution, resulting in decreased I\(_{Na}\).

After rapid activation and inactivation, the membrane potential is far removed from the Na\(_v\) activation threshold, and Na\(_v\) recovery from inactivation is needed. Our results show that the Na\(_v\) recovery time from inactivation to activation is not affected by either ischemic solution or EA serum, suggesting that neither has a significant effect on Na\(_v\). recovery from inactivation.
In conclusion, ischemia increases $I_{Na}$ by delaying $Na_+$ inactivation and broadening the $Na_+$ activation interval, which can be restored by EA at bilateral Neiguan (PC 6). Our study suggests that EA at bilateral Neiguan (PC 6) may attenuate $Na_+$ overload and promote functional recovery in ischemic ventricular myocytes.

ACKNOWLEDGMENTS

We would like to thank Prof. Yiguo Chen of Liaoning University of Traditional Chinese Medicine for the work on this article.

REFERENCES