Electrophysiological effects of resveratrol on guinea pig papillary muscles

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Abstract: The purpose of this study was to investigate the electrophysiological effects of resveratrol on guinea pig papillary muscles and the underlying mechanism. Action potentials were recorded by using intracellular microelectrode technique. The results obtained are as follows: (1) In normal papillary muscles, resveratrol (30, 60, and 120 µmol/L) shortened the duration of action potential (APD) in a concentration-dependent manner. (2) In partially depolarized papillary muscles, resveratrol (60 µmol/L) not only shortened APD, but also decreased the amplitude of action potential (APA), overshoot (OS) and maximal rate of depolarization in phase 0 (V\text{max}). (3) Perfusion with Ca\textsuperscript{2+}-free K-H solution, completely abolished the effects of resveratrol (60 µmol/L) on papillary muscles. (4) Application of potassium channel blocker tetraethylammonium chloride (TEA, 20 mmol/L) did not prevent the effect of resveratrol (60 µmol/L) on action potential. (5) Pretreatment with L-nitro-arginine methyl ester (L-NAME, 1 mmol/L), a nitric oxide (NO) synthase inhibitor, failed to abolish the effect of resveratrol (60 µmol/L). All these results indicate that the electrophysiological effects of resveratrol on guinea pig papillary muscles are likely due to the reduction of calcium influx, which might not be mediated by NO.

Key words: electrophysiology; resveratrol; action potential; papillary muscle; calcium current

白藜芦醇对离体豚鼠乳头状肌的电生理效应

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摘 要：本文旨在应用标准玻璃微电极技术，观察白藜芦醇对离体豚鼠乳头状肌的电生理效应。结果显示：(1)白藜芦醇(30、60、120 µmol/L)可剂量依赖性地缩短乳头状肌细胞的动作电位时程；(2)对部分去极化的乳头状肌，白藜芦醇(60 µmol/L)不仅缩短动作电位时程，而且降低动作电位的幅值和超射值，减慢零期最大上升速度；(3)用无钙K-H液灌流标本可完全取消白藜芦醇对乳头状肌细胞的作用；(4)钾通道开放剂四乙基氯化铵(TEA, 20 mmol/L)，不能阻断白藜芦醇的电生理效应；(5)预先应用一氧化氮合酶抑制剂L-NAME(1 mmol/L)，对白藜芦醇的上述效应无影响。以上结果表明，白藜芦醇可缩短正常乳头状肌细胞动作电位时程，这一效应可能与其抑制钙离子内流有关，但此作用机制中NO的作用并不显著。

关键词：电生理；白藜芦醇；动作电位；乳头状肌；钙电流

中图分类号：Q463

Resveratrol (trans-3,4’,5-trihydroxystilbene), a common polyphenol phytoalexin, is abundant in some Chinese herbal medicine, especially in rhizoma polygoni cuspidate. Resveratrol also exists in a few edible materials, such as grape, peanuts, and red wine. Resveratrol possesses many biochemical and physiological actions, including estrogenic, anti-oxidant, anti-inflammatory, and anti-cancer properties [1-4]. Accumulating line of evidence supported the view that resveratrol may exert protective action on the cardiovascular system. Resveratrol could protect the heart from ischemia-reperfusion injury [5]. It decreased plasma triglycerides and cholesterol accumulation in the aorta and prevented atherosclerosis [6]. Moreover, it relaxed the coronary arteries [7,8], inhibited platelet aggregation [9]. Thus, resveratrol may have a potential clinical value in the treatment of cardiovascular diseases. However, little is known...
about the electrophysiological effects of resveratrol on cardiomyocytes. The aim of the present study was to investigate the effects of resveratrol on the action potential of guinea pig papillary muscles and the underlying mechanism.

1 MATERIALS AND METHODS

1.1 Preparation. Male guinea pig (n=30, weighing 250–350 g, provided by Experimental Animal Center of Hebei Province) was killed by a heavy blow on the head and the heart was quickly removed and placed in cold (0–4°C) Krebs-Henseleit (K-H) solution. The papillary muscle was excised from right ventricle and pinned down on a thin silicon rubber disc placed on the bottom of a perfusion chamber by stainless steel needles. The preparation was perfused (4 ml/min) with K-H solution containing the following compositions (mmol/L): NaCl 118.0, NaHCO3 25.0, KCl 4.7, MgSO4 1.6, CaCl2 2.5, KH2PO4 1.2, and glucose 11.1. The K-H solution was oxygenated with 95% O2 and 5% CO2 and maintained at (35.5 ± 0.5)ºC with pH of 7.38 ± 0.03.

1.2 Electrophysiological recording. The papillary muscle was paced by square pulse (1 Hz, 1 ms, 1.5 times threshold) generated by an electronic stimulator (SEN-3201, Nihon Kohden). The transmembrane action potentials were recorded with a glass microelectrode filled with 3 mol/L KCl (a tip resistance of 10–20 MΩ), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D converter and processed by a microcomputer. Resting potential (RP), overshoot (OS), amplitude of action potential (APA), maximal rate of depolarization in phase 0 (Vmax), duration of 50% repolarization (APD50), duration of 90% repolarization (APD90), duration of action potential (APD) were analyzed, and duration of plateau phase (PPD) was calculated from regression analysis on repolarization of phase 2 and phase 3[10].

1.3 Experimental protocols. The experiments started after the preparations were equilibrated for 60 min in K-H solution. The action potentials (AP) were recorded before and at 1, 5, 10, 15, 20, 25 and 30 min after application of drugs. The experiments consisted of 5 groups: (1) Electrophysiological effects of resveratrol on normal papillary muscles: After recording of 3 control AP, resveratrol 30, 60, and 120 µmol/L were applied respectively, and then AP were recorded. (2) Electrophysiological effects of resveratrol on partially depolarized papillary muscles: The preparations were equilibrated for 60 min in normal K-H solution firstly. Then slow response AP was induced in the papillary muscles through exposure to K-H solution containing 18 mmol/L KCl and 1.5 µmol/L isoproterenol. After recording of 3 control AP, resveratrol (60 µmol/L) was administered and AP were recorded. (3) Effects of resveratrol on AP in normal papillary muscles in Ca2+-free K-H solution: The effects of resveratrol (60 µmol/L) alone were observed firstly. Then after superfusion with Ca2+-free K-H solution instead of normal K-H solution for 60 min, resveratrol 60 µmol/L was added and AP were recorded. (4) Effects of tetraethylammonium chloride (TEA) on the action of resveratrol: The effects of resveratrol (60 µmol/L) alone were observed firstly. Then upon pretreatment with TEA (20 µmol/L) for 10 min, resveratrol 60 µmol/L was added to the superfusate and AP were recorded. (5) Effects of Nω-nitro-L-arginine methyl ester (L-NAME) on the response of papillary muscles to resveratrol: The effects of resveratrol (60 µmol/L) were observed before and after superfusion of L-NAME (1mmol/L) for 10 min. In each experiment, the preparation was washed out with K-H solution after application of drugs to observe the recovery of AP.

1.4 Drugs. Drugs used in this study included resveratrol, TEA and L-NAME (Sigma). Resveratrol was dissolved in DMSO. The final concentration of DMSO was 0.05%. TEA and L-NAME were dissolved in distilled water.

1.5 Statistics. All data were expressed as mean±SE. Statistical analyses were evaluated by one-way ANOVA and t test. Statistical significance was accepted at P<0.05.

2 RESULTS

2.1 Effects of resveratrol on action potential in normal guinea pig papillary muscles

In normal papillary muscles, resveratrol (30, 60, and 120 µmol/L) shortened PPD, APD50, APD90 and APD in a concentration-dependent manner. These effects occurred after 5 min perfusion with resveratrol and reached the peak within 15–20 min. Resveratrol (30 µmol/L) had no significant effect on the parameters of AP. While resveratrol (60 and 120 µmol/L) significantly shortened PPD, APD50, APD90 and APD, it had no effect on RP, OS, APA and Vmax (Fig. 1.4, Table 1). The vehicle of resveratrol (0.05% DMSO in superfusate) showed no effect on the parameters of AP in papillary muscles.

2.2 Effects of resveratrol on action potential in par-
Perfusion with K-H solution containing 18 mmol/L KCl and 1.5 µmol/L isoprenaline resulted in partial depolarization in papillary muscles. Under this condition, resveratrol (60 µmol/L) not only shortened PPD, APD50, APD90, and APD, but also decreased OS, APA, and Vmax (Fig.1B, Table 2).

2.3 Effects of resveratrol on normal papillary muscles in Ca2+-free K-H solution

Superfusion with Ca2+-free K-H solution instead of normal K-H solution significantly shortened PPD, APD50, APD90, and APD, and decreased OS and APA. After equilibration for 60 min, 60 µmol/L resveratrol was added. In Ca2+-free superfusate, resveratrol failed to affect AP in normal papillary muscles (Table 3).

2.4 Effects of TEA on the action of resveratrol in normal papillary muscles

Potassium channel antagonist TEA (20 mmol/L) markedly increased PPD, APD50, APD90, and APD, but had no effect on other parameters. Upon the administration of TEA for 10 min, resveratrol (60 µmol/L) still decreased PPD, APD50, APD90, and APD (Table 3).

2.5 Effects of L-NAME on the response of normal papillary muscles to resveratrol

NO synthase inhibitor L-NAME (1 mmol/L) itself had no significant effect on AP. Pretreatment with 1 mmol/L L-NAME for 10 min did not influence the effects of resveratrol (60 µmol/L) on AP (Table 3).

3 DISCUSSION

The present study demonstrated that resveratrol concentration-dependently shortened PPD, APD50, APD90, and APD of action potential in normal guinea pig papillary muscles. The duration of action potential is determined by the duration of repolarization, which is mainly influenced by calcium influx and potassium efflux. So either factor inhibiting calcium influx or promoting potassium efflux may shorten APD. In partially depolarized papillary muscles induced by exposure to 18 mmol/L KCl and 1.5 µmol/L

<table>
<thead>
<tr>
<th>Table 1. Effects of resveratrol on action potential in normal guinea pig papillary muscles (n = 6)</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>30 µmol/L resveratrol</td>
</tr>
<tr>
<td>60 µmol/L resveratrol</td>
</tr>
<tr>
<td>120 µmol/L resveratrol</td>
</tr>
</tbody>
</table>

NO synthase inhibitor L-NAME (1 mmol/L) itself had no significant effect on AP. Pretreatment with 1 mmol/L L-NAME for 10 min did not influence the effects of resveratrol (60 µmol/L) on AP (Table 3).

3 DISCUSSION

The present study demonstrated that resveratrol con-
isoprenaline, the slow response AP with low amplitude and low rate of depolarization was elicited\cite{11, 12}. To test the calcium channel blocker nifedipine was administered. We observed that nifedipine could completely inhibit the slow response AP, suggesting that calcium influx instead of sodium influx plays an important role in the depolarization of slow response AP. In partially depolarized papillary muscles, resveratrol not only shortened PPD, APD<sub>50</sub>, APD<sub>90</sub> and APD<sub>ms</sub>, but also reduced OS, APA, and \( V_{\text{max}} \) in phase 0, a calcium channel blocker nifedipine was administered in our experiment. We observed that nifedipine failed to abolish the effects of resveratrol. Therefore, we speculated that in guinea pig papillary muscles, NO might not be involved in the electrophysiological effects of resveratrol, and the mechanism need further investigation.

In conclusion, resveratrol shortened APD in normal guinea pig papillary muscles and decreased OS, APA, \( V_{\text{max}} \) and APD in partially depolarized papillary muscles. The electrophysiological effects of resveratrol are likely due to a decrease of calcium influx via a NO-independent mechanism. Our study offers the electrophysiological basis for the application of resveratrol to treatment of cardiovascular diseases.

### Table 3. Influences of Ca\(^{2+}\)-free, TEA (20 mmol/L) and L-NAME (1 mmol/L) on the resveratrol (60 \( \mu \)mol/L)-induced changes on action potential in guinea pig papillary muscles (n=18)

<table>
<thead>
<tr>
<th></th>
<th>RP(mV)</th>
<th>OS(mV)</th>
<th>APA(mV)</th>
<th>( V_{\text{max}} )(V/s)</th>
<th>PPD(ms)</th>
<th>APD&lt;sub&gt;50&lt;/sub&gt;(ms)</th>
<th>APD&lt;sub&gt;90&lt;/sub&gt;(ms)</th>
<th>APD&lt;sub&gt;ms&lt;/sub&gt;(ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(-85.1\pm0.9)</td>
<td>(28.9\pm1.6)</td>
<td>(114.0\pm2.2)</td>
<td>(216.4\pm19.4)</td>
<td>(102.8\pm6.2)</td>
<td>(135.6\pm7.5)</td>
<td>(160.6\pm5.9)</td>
<td>(175.1\pm6.1)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>(-85.0\pm0.9)</td>
<td>(28.4\pm1.7)</td>
<td>(113.5\pm2.3)</td>
<td>(216.1\pm20.0)</td>
<td>(90.5\pm7.3^{**})</td>
<td>(120.8\pm8.8^{***})</td>
<td>(146.6\pm7.4^{*})</td>
<td>(161.2\pm7.6^{*})</td>
</tr>
<tr>
<td>Ca(^{2+})-free</td>
<td>(-84.3\pm1.1)</td>
<td>(23.5\pm1.1^{**})</td>
<td>(107.6\pm1.6^{**})</td>
<td>(215.4\pm19.2)</td>
<td>(23.6\pm3.5^{**})</td>
<td>(40.2\pm4.2^{***})</td>
<td>(65.4\pm8.3^{*})</td>
<td>(78.2\pm8.7^{*})</td>
</tr>
<tr>
<td>Ca(^{2+})-free+resveratrol</td>
<td>(-84.4\pm1.0)</td>
<td>(23.4\pm1.0^{**})</td>
<td>(107.6\pm1.6^{**})</td>
<td>(215.6\pm19.3)</td>
<td>(23.2\pm3.9^{**})</td>
<td>(39.8\pm4.7^{***})</td>
<td>(65.2\pm8.0^{*})</td>
<td>(77.4\pm8.6^{*})</td>
</tr>
<tr>
<td>Control</td>
<td>(-86.4\pm0.8)</td>
<td>(32.5\pm2.4)</td>
<td>(118.9\pm2.8)</td>
<td>(210.4\pm18.4)</td>
<td>(105.6\pm3.8)</td>
<td>(139.0\pm4.5)</td>
<td>(164.6\pm5.4)</td>
<td>(181.0\pm5.2)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>(-85.9\pm1.1)</td>
<td>(32.2\pm2.5)</td>
<td>(118.1\pm2.9)</td>
<td>(209.0\pm17.4)</td>
<td>(93.8\pm3.8^{***})</td>
<td>(124.8\pm4.6^{***})</td>
<td>(150.4\pm5.4^{***})</td>
<td>(166.6\pm5.1^{***})</td>
</tr>
<tr>
<td>TEA</td>
<td>(-86.0\pm0.9)</td>
<td>(31.8\pm1.9)</td>
<td>(117.8\pm2.5)</td>
<td>(209.3\pm18.1)</td>
<td>(119.0\pm4.0^{*})</td>
<td>(155.2\pm4.8^{**})</td>
<td>(182.4\pm3.3^{**})</td>
<td>(200.6\pm4.4^{**})</td>
</tr>
<tr>
<td>TEA+resveratrol</td>
<td>(-86.1\pm0.8)</td>
<td>(31.4\pm1.8)</td>
<td>(117.2\pm2.4)</td>
<td>(209.8\pm17.9)</td>
<td>(105.6\pm4.6^{*})</td>
<td>(139.4\pm5.3^{*})</td>
<td>(169.1\pm4.3^{*})</td>
<td>(183.2\pm4.4^{*})</td>
</tr>
<tr>
<td>Control</td>
<td>(-86.1\pm0.8)</td>
<td>(32.3\pm0.2)</td>
<td>(118.4\pm0.8)</td>
<td>(221.7\pm11.7)</td>
<td>(94.0\pm5.5)</td>
<td>(125.0\pm6.6)</td>
<td>(154.0\pm7.2)</td>
<td>(168.3\pm6.9)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>(-85.6\pm1.1)</td>
<td>(31.9\pm0.3)</td>
<td>(117.5\pm1.2)</td>
<td>(222.3\pm10.7)</td>
<td>(82.6\pm6.0^{***})</td>
<td>(111.3\pm7.2^{***})</td>
<td>(140.3\pm7.5^{***})</td>
<td>(153.7\pm7.4^{***})</td>
</tr>
<tr>
<td>L-NAME</td>
<td>(-85.3\pm0.6)</td>
<td>(32.5\pm0.6)</td>
<td>(117.8\pm0.8)</td>
<td>(220.3\pm12.4)</td>
<td>(95.5\pm6.1)</td>
<td>(126.8\pm7.4)</td>
<td>(155.8\pm7.7)</td>
<td>(169.8\pm7.7)</td>
</tr>
<tr>
<td>L-NAME+resveratrol</td>
<td>(-85.4\pm0.6)</td>
<td>(32.5\pm0.4)</td>
<td>(117.9\pm0.8)</td>
<td>(219.3\pm10.3)</td>
<td>(84.3\pm6.8^{***})</td>
<td>(113.3\pm8.2^{***})</td>
<td>(142.8\pm8.5^{***})</td>
<td>(155.7\pm7.6^{***})</td>
</tr>
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</table>

\( ^{**}P<0.01, ^{***}P<0.001 \) vs control. \( ^{*}P<0.01, ^{*}\cdots^{*}P<0.001 \) vs TEA. \( ^{**}P<0.001 \) vs L-NAME.

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