Electrophysiological effect of resveratrol on pacemaker cells in sinoatrial node of rabbits

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Abstract: **AIM** In order to investigate whether resveratrol can be used as a kind of antiarrhythmic drug, the electrophysiological effect of resveratrol on pacemaker cells in sinoatrial node was studied. **METHODS** Using intracellular microelectrode technique to record the action potential of pacemaker cells in sinoatrial node of rabbits. **RESULTS** Resveratrol (30 – 120 $\mu mol \cdot L^{-1}$) significantly decreased amplitude of action potential, maximal rate of depolarization (V_{max}), velocity of diastolic (phase 4) depolarization and rate of pacemaker firing, but did not affect maximal diastolic potential and duration of 90% repolarization of action potential. Pretreatment with L-type calcium channel agonist Bay-K-8644 (0.5 $\mu mol \cdot L^{-1}$) 10 min antagonized the effect of resveratrol (60 $\mu mol \cdot L^{-1}$). While applying cesium chloride (2 $mmol \cdot L^{-1}$), a hyperpolarization-activated current blocker, adding tetraethylammonium chloride (20 $mmol \cdot L^{-1}$), a potassium channel antagonist, or applying *L*-NAME (0.5 $mmol \cdot L^{-1}$), a NO synthase inhibitor, had no significantly influence on the electrophysiological effects of resveratrol. **CONCLUSION** Resveratrol exerts inhibitory electrophysiological effects on pacemaker cells in sinoatrial node of rabbits, which may be due to reduction in calcium influx *via* a NO-independent manner.

Key words: resveratrol; sinoatrial node; calcium channels; action potentials; microelectrodes

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Resveratrol (trans-3, 4′, 5-trihydroxystilbene) is a phenolic phytoalexin present in grape skins and wines, especially red wines. Resveratrol exerts a wide variety of biological effects, including estrogenic, anti-inflammatory, antioxidant and anticancer activities[1-4]. Moreover, accumulating evidence indicates that resveratrol may confer protective action on the cardiovascular system. The cardiovascular benefits of resveratrol may relate to protecting the heart cells from ischemia-reperfusion injury, inhibiting platelet aggregation, and decreasing plasma triglycerides and cholesterol accumulation in the aorta[5-7]. Furthermore, it can also relax the coronary arteries[8]. Thus, resveratrol may have a potential clinical value in treatment of cardiovascular diseases. In our previous studies, we found that resveratrol shortened the duration of action potential in papillary muscles in normal guinea pig and also decreased the maximal velocity of phase 0 depolarization in partially depolarized papillary muscles. These effects were likely due to a decrease in calcium influx[9]. Little is known, however, about the effects of resveratrol on pacemaker cells of isolated sinoatrial node. In the present study, we observed the electrophysiological effects of resveratrol on sinoatrial node cells of rabbits and investigated the mechanism involved.

1 MATERIALS AND METHODS

1.1 Drugs

Drugs used in this study included resveratrol, Bay-K-8644, tetraethylammonium chloride (TEA), N^G-nitro-*L*-arginine methyl ester (*L*-NAME) and CsCl (Sigma Chemical Co, USA). Resveratrol was prepared as stock solution in DMSO and final concentration of DMSO was less than 0.1%. Bay-K-8644 was prepared as stock solution in alcohol and the final concentration of alcohol was 0.1%. CsCl, TEA and *L*-NAME

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were dissolved in distilled water.

1.2 Sinoatrial node preparations

Rabbit (♂, $n = 24$, weighing 2.2 – 2.8 kg, Grade Ⅱ, Certificate №. 04037, provided by Experimental Animal Center of Hebei Province) was sacrificed with a single blow on head and the heart was removed and placed in cold (0 – 4℃) oxygenated (95% O_2 and 5% CO_2) Krebs-Henseleit (K-H) solution. The region of the right atrium bounded by the crista terminalis and the superior and inferior vena cava, and the interatrial septum was dissected free from the adjacent tissue[10]. The preparation was fixed with fine pins to the silica gel on the base of a perfusion chamber.

1.3 Superfusing solution

The K-H solution was prepared with deionized, distilled water and had the following compositions (mmol·L^{-1}): NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, and glucose 11.1. It was oxygenated with 95% O_2 and 5% CO_2 and maintained at 35.5 – 36.5 ℃ with pH 7.35 – 7.40.

1.4 Electrical recording

The transmembrane action potentials (AP) were recorded from pacemaker cells by means of 3 mol·L^{-1} KCl-filled microelectrode (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. Maximal diastolic potential (MDP), amplitude of action potential (APA), maximal rate of depolarization (V_{max}), velocity of diastolic (phase 4) depolarization (VDD), rate of pacemaker firing (RPF) and duration of 90% repolarization of action potential (APD_{90}) were analyzed with a program of sampling and processing cardiac transmembrane potential designed by our department[11]. Parameters were stored in the microcomputer for later analysis.

1.5 Experimental protocols

The experiment started after the preparation was equilibrated for 60 min in the K-H solution at a perfusion rate of 4 mL·min^{-1}. The experiments consisted of 2 parts: ① Electrophysiological effects of resveratrol on sinoatrial node pacemaker cells. After recording of 3 control AP, resveratrol 30, 60, 120 μmol·L^{-1} were applied in turn, and resveratrol was washed off before the next dose. AP were then recorded at 1, 5, 10, 15 and 20 min after application of each concentration of resveratrol. ② Effects of Bay-K-8644, CsCl + TEA or *L*-NAME on the response of pacemaker cells to resveratrol. The effects of resveratrol (60 μmol·L^{-1}) alone were observed firstly after application for 15 min. Then, the preparation was washed out with K-H solution until the parameters restored to control level. At last, the preparations were superfused with K-H solution containing 0.5 μmol·L^{-1} Bay-K-8644, 2 mmol·L^{-1} CsCl + 20 mmol·L^{-1} TEA or 0.5 mmol·L^{-1} *L*-NAME for 10 min, and final resveratrol (60 μmol·L^{-1}) was added to the superfusate containing Bay-K-8644, CsCl + TEA or *L*-NAME and AP were recorded.

1.6 Statistical analysis

All data were presented as $\bar{x} \pm s$. The differences in the parameters between before and after resveratrol perfusion were compared by paired *t* test. Differences between groups were assessed by unpaired *t* test.

2 RESULTS

2.1 Effects of resveratrol on transmembrane action potentials

Compared with control group, resveratrol (30, 60 and 120 μmol·L^{-1}) decreased APA, V_{max}, VDD, and RPF in a concentration-dependent manner (Fig 1, Tab 1). The changes in RPF induced by resveratrol paralleled to those of VDD. The above effects occurred after 5 min of superfusion of resveratrol and reached the peak within 10 – 15 min. The vehicle of resveratrol (0.1% DMSO in superfusate) had no effect on parameters of AP of pacemaker cells.

2.2 Effect of Bay-K-8644, CsCl + TEA or *L*-NAME on resveratrol-induced changes in action potential

L-type calcium channel agonist Bay-K-8644 (0.5 μmol·L^{-1}) significantly increased APA,

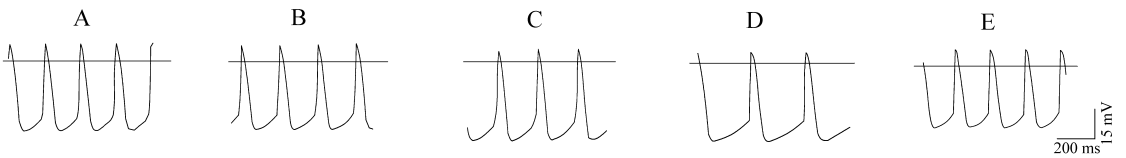

Fig 1. Original recording showing effect of resveratrol on transmembrane action potentials (AP) of rabbit sinoatrial node cells. AP were recorded at 15 min after application of resveratrol. A: control; B, C and D: resveratrol 30, 60 and 120 μmol·L^{-1}, respectively; E: wash out.

Tab 1. Effect of resveratrol on transmembrane action potentials(AP) of rabbit sinoatrial node cells

Resveratrol /μmol·L^{-1}	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/min^{-1}	APD_{90}/ms
0	(−54.2±6.7)	(68.8±6.5)	(8.1±0.8)	(61.2±6.5)	(168.1±15.9)	(156.3±12.7)
30	−1.2±0.4	−2.0±0.8*	−0.3±0.5*	−3.8±3.9**	−12.3±4.1	−0.1±3.6
60	−0.5±0.4	−3.8±2.3**	−1.7±1.2**	−20.9±12.7**	−26.0±11.2**	0.7±4.2
120	−1.3±0.3	−6.2±2.2**	−1.9±1.2**	−27.6±19.5**	−59.0±31.3**	2.2±5.1

AP were recorded at 15 min after application of resveratrol 30, 60 and 120 μmol·L^{-1} in turn, and resveratrol was washed off before the next dose. The results given are differences between control and a given group, and those bracketed in control group are original readings. MDP: maximal diastolic potential; APA: amplitude of action potential; V_{max}: maximal rate of depolarization; VDD: velocity of diastolic (phase 4) depolarization; RPF: rate of pacemaker firing; APD_{90}: duration of 90% repolarization of action potential. $\bar{x} \pm s$, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control group by paired t test.

Tab 2. Influences of Bay-K-8644, CsCl + TEA and *L*-NAME on resveratrol-induced effects on transmembrane action potentials(AP) of rabbit sinoatrial node cells

Group	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/min^{-1}	APD_{90}/ms
Control	(−51.6±5.2)	(58.7±5.9)	(6.1±0.8)	(49.4±8.5)	(165.8±26.7)	(166.2±25.4)
Resveratrol	−0.1±0.2	−2.8±1.7**	−0.9±0.4**	−9.7±5.5**	−30.3±10.4**	−1.3±2.7
Bay-K-8644	0.0±0.6	3.0±1.9*	1.2±0.5*	14.7±4.2**	29.6±13.3**	1.2±2.5
Bay-K-8644 + resveratrol	−0.1±0.2	−2.1±2.0*	0.2±0.5$^{\#}$	−0.4±2.0$^{\#}$	−1.9±16.1$^{\#}$	1.3±1.5
Control	(−56.0±6.6)	(63.1±8.4)	(4.9±2.1)	(46.8±12.7)	(187.7±18.8)	(162.7±10.0)
Resveratrol	0.2±0.5	−2.4±2.2*	−1.6±0.5**	−7.9±4.2**	−23.9±9.0**	−0.9±4.0
CsCl + TEA	−1.8±0.7**	−0.2±0.3	−0.3±0.3	−15.5±8.8**	−34.1±11.0**	22.8±15.6*
CsCl + TEA + resveratrol	−1.8±0.7$^{**}_{\#\#}$	−2.5±1.2**	−1.6±0.8**	−21.1±5.8$^{**}_{\#\#}$	−56.3±7.9$^{**}_{\#\#}$	25.7±17.8$^{*}_{\#\#}$
Control	(−48.4±4.4)	(64.1±2.3)	(7.3±0.3)	(57.2±7.9)	(176.8±16.4)	(143.9±18.8)
Resveratrol	−0.2±0.6	−3.5±0.8**	−1.3±0.9*	−11.9±5.6*	−42.8±9.0**	2.1±2.6
L-NAME	−0.3±1.0	−0.1±0.5	−0.1±0.3	−0.9±1.4	−3.2±3.7	1.8±2.4
L-NAME + resveratrol	0.1±0.5	−4.1±1.8**	−1.7±0.5*	−10.8±6.8*	−38.6±12.7**	4.3±3.1

AP were recorded before (control) and after perfusing with resveratrol 60 μmol·L^{-1} for 15 min. After washing out until parameters of the pacemaker cells restored to control level, sinoatrial nodes were perfused with K-H solution containing 0.5 μmol·L^{-1} Bay-K-8644, 2 mmol·L^{-1} CsCl + 20 mmol·L^{-1} TEA or 0.5 mmol·L^{-1} *L*-NAME for 10 min and AP were recorded respectively. Then 60 μmol·L^{-1} resveratrol was added in the presence of one of mentioned drugs, and AP were recorded 15 min later again. $\bar{x} \pm s$, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with corresponding control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with corresponding resveratrol group.

V_{max}, VDD and RPF. Upon the application of Bay-K-8644, the effect of resveratrol (60 μmol·L^{-1}) was abolished (Tab 2). The vehicle of Bay-K-8644 (0.1% alcohol in superfusate) had no effect on parameters of AP of pacemaker cells.

After application of 2 mmol·L^{-1} CsCl and 20 mmol·L^{-1} TEA into the superfusate, VDD and RPF of AP of sinoatrial node cells were significantly decreased. Pretreatment with CsCl and TEA failed to block the effects of resveratrol (60

μmol·L^{-1}) (Tab 2).

NO synthase inhibitor *L*-NAME (0.5 mmol·L^{-1}) *per se* had no effect on AP. Upon the administration of *L*-NAME (0.5 mmol·L^{-1}) for 10 min, resveratrol (60 μmol·L^{-1}) still decreased APA, V_{max}, VDD and RPF (Tab 2).

3 DISCUSSION

The present study demonstrated that resveratrol exerted inhibitory actions on the automaticity of pacemaker cells in sinoatrial node of rabbits, and decreased APA, V_{max}, VDD and RPF in a concentration-dependent manner. It has been widely accepted that calcium currents play important roles in AP upstroke and pacemaker depolarization of sinoatrial node cells[12,13]. Therefore, the inhibitory effects of resveratrol on APA, V_{max}, VDD and RPF may be attributed to the reduction of I_{Ca}. Our presumption was substantiated by the following findings that application of *L*-type Ca^{2+} channel agonist Bay-K-8644 abolished the inhibitory effects of resveratrol.

There is a question that why resveratrol could reduce the APD_{90} of guinea pig papillary muscle cells[9] while not reduce that of pacemaker cells in sinoatrial node of rabbits. This may ascribe to difference of AP of the two kinds of cells. There is a long plateau in the repolarization of the papillary muscle cells AP, while not in the pacemaker cells AP.

The duration of AP is determined by the duration of repolarization. On the papillary muscle cells, the duration of repolarization depends on the duration of plateau which is mainly influenced by calcium influx and potassium efflux. Resveratrol could inhibit the influx of the calcium current and result in the short of the APD_{90} of papillary muscle cells. While on the pacemaker cells in sinoatrial node of rabbits, resveratrol mainly affect the phase 0 of the AP and this may be the reason why it could not significantly reduce the APD_{90} of pacemaker cells.

It is well known that three kinds of ion currents (I_k, I_f, I_{Ca}) were involved in the diastolic depolarization of the sinoatrial node cells[12]. Li, *et al*[14] reported that resveratrol directly stimulated Ca^{2+}-activated K^+ current in vascular endothelial cells. To investigate whether potassium current and hyperpolarization-activated current were involved in the effects of resveratrol on pacemaker cells, potassium ion channel blocker TEA and I_f antagonist CsCl were employed. In our experiments, administration of TEA and CsCl could not affect the action of resveratrol, suggesting that resveratrol do not influence potassium current and hyperpolarization-activated current in pacemaker cells. This result implies that the effects of resveratrol on AP of sinoatrial node cells are probably due to the reduction of I_{Ca}.

A growing number of studies imply that the role of NO production was involved in some effects of resveratrol[5]. From this point of view, resveratrol could induce an increase in the production of NO, thereby resulting in a rise in intracellular cGMP with a subsequent reduction in intracellular calcium[15]. On the contrary, Dobrydneva, *et al*[16] reported that resveratrol has direct effect on calcium channel through a NO-independent pathway in human platelets. In order to elucidate whether NO is involved in the inhibitory effects of resveratrol on calcium influx in pacemaker cells, *L*-NAME, a NO synthase inhibitor, was administered in our study. We observed that *L*-NAME failed to abolish the effects of resveratrol. Therefore, we speculated that on pacemaker cells in sinoatrial node, NO may not be involved in the electrophysiological effects of resveratrol, and the mechanism remains to be further established.

However, we can not rule out whether resveratrol exerts its effects through some other unknown secondary messenger, and whether the T-type calcium current was involved in the effects of resveratrol and this issue merits further investigation by patch-clamp technique.

In summary, resveratrol exhibited inhibitory effects on pacemaker cells in sinoatrial node of rabbits. These effects are probably due to reduction in calcium influx *via* a NO-independent mechanism.

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白藜芦醇对家兔窦房结起搏细胞的电生理效应

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摘要：目的　为探讨白藜芦醇是否能成为抗心律失常药，研究了其对窦房结起搏细胞的电生理效应。**方法**　应用细胞内微电极方法记录家兔窦房结起搏细胞的动作电位。**结果**　白藜芦醇(30 ~ 120 $\mu mol \cdot L^{-1}$)显著降低窦房结起搏细胞的动作电位幅度、零相最大上升速率(V_{max})、舒张期除极速率和起搏放电频率。而对最大舒张期电位和 90% 复极化的时间无明显作用。预先应用 L 型钙通道开放剂 Bay-K-8644(0.5 $\mu mol \cdot L^{-1}$)灌流窦房结 10 min 可阻断白藜芦醇(60 $\mu mol \cdot L^{-1}$)对起搏细胞的上述电生理效应。而应用超极化激活电流阻断剂氯化铯(2 $mmol \cdot L^{-1}$)加钾通道阻断剂四乙铵(20 $mmol \cdot L^{-1}$)或应用一氧化氮(NO)合酶阻断剂 *L*-NAME(0.5 $mmol \cdot L^{-1}$)灌流窦房结标本 10 min 对白藜芦醇(60 $\mu mol \cdot L^{-1}$)的电生理效应没有明显影响。**结论**　白藜芦醇能抑制家兔窦房结起搏细胞的自发活动，此效应可能与其通过非 NO 依赖性途径抑制钙离子内流有关。

关键词： 白藜芦醇；窦房结；钙通道；动作电位；微电极

（本文编辑　董立春）