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The vasorelaxing effect of resveratrol on abdominal aorta from rats and its underlying mechanisms

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ABSTRACT

Evidence has indicated that resveratrol (Res) produces vasorelaxation and may decrease the coronary heart disease mortality. However, several pathways involved in the mechanism of vasorelaxation are still unclear. This study was designed, therefore, to test the probable ion channels or receptors involved in the mechanism. The abdominal aortic rings from the male Sprague-Dawley rats were perfused in the organ chambers filled with Kreb's solution, where the tension of each ring was measured. Treatment with L-NAME (a nitric oxide synthase inhibitor), glibenclamide and tetraethylammonium (TEA) significantly attenuated the vasorelaxing effect of Res. In lower concentration Res relaxed the ring in an endothelium-dependent manner, while in higher concentration the endothelium-independent manner could be observed. In calcium-free Kreb's solution, Res inhibited vasoconstriction induced by NE. With intracellular calcium depleted by thapsigargin, Res also inhibited vasoconstriction induced by Kreb's solution with high potassium via L-Ca^{2+} channel. In a word, Res decreased both extracellular calcium influx and intracellular calcium release. These results suggest that: (1) Res may exert its relaxing effect on abdominal aorta by opening K^+ channel to hyperpolarize vascular smooth muscle.(2) Res relaxes the abdominal aorta in both endothelium-dependent and endothelium-independent manners. (3) Finally, Res attenuates both extracellular calcium influx and intracellular calcium release, which results in vasorelaxation.

1. Introduction

Res is a polyphenolic compound which mainly exists in red grapes and wine. In a previous epidemiological study (Renaud and de Lorgeril, 1992) on the relationship between eating habits and coronary heart disease (CHD), an interesting phenomenon suggests that in all developed countries, the French consume the most quantity of wine in average, but have the lowest CHD morbidity. This phenomenon is called "French paradox", which has probably benefited from Res in wine. Previous studies have also demonstrated that Res plays a role in preventing cancer (Aluyen et al., 2012), modulating inflammation response (Rahal et al., 2012; Chan et al., 2011) and protecting neurons (Li et al., 2012; Lopez-Miranda et al., 2012). Moreover, there have been additional studies implicating the vasorelaxing effect of Res (Bertini et al., 2010).

As has been widely accepted Res has a vasorelaxing effect. Naderali et al. (2000) found that the relaxing effect of Res on the mesenteric (resistance) artery was greater than that on the uterine (conductance) artery. And their study also displayed that the relaxing effect was not mediated via prostanoids, but NO could play a role. In another study, Naderali et al. (2001) discovered that the vasorelaxing effect of Res in lean animals (where endothelial function is not impaired), but not in dietary-obese rats, were mediated via NO, and there were no significant differences between the two groups, achieving the same maximum relaxation of >95% irrespective of the endothelial function.

Studies also demonstrated that NO-GC-cGMP pathway plays an important regulatory role in the vasorelaxing effect of Res. El-Mowafy (2002) recently discovered that the underlying mechanism of coronary-protective effect of Res was closely related to the membrane-bound guanylyl cyclase (GC) in coronary arterial smooth muscle. Res might elevate the cGMP level in coronary artery which was due to the activation of GC in the particulate (pGC) but not in the soluble GC (sGC). This pathway triggered vasorelaxing responses in both intact endothelial and endothelium-disrupted arteries. Besides, Li et al. (2000) discovered large-conductance Ca^{2+}-activated-K^+ current ($I_{K,Ca}$) mediated by BK_{Ca}

Abbreviations: Res, Resveratrol; CHD, Coronary heart disease; BK_{Ca}, Large-conductance $Ca2^{+}$-activated K^+; L-NAME, N(G)-nitro-L-arginine methyl ester; eNOS, Endothelial NO synthase; TEA, Tetraethylammonium; Gly, Glibenclamide; AT, Atropine; PPL, Propranolol; IMT, Indometacin.

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was increased when treated with Res. The opening time of BK_{Ca} was prolonged by Res in a dose-dependent manner. Moreover, Res increases the activity of intermediate-conductance Ca^{2+}-activated-K^+ current, which is probably associated with endothelial function. It is widely acknowledged that calcium is a key factor in vasoconstriction. However, the relationship between calcium and Res is still unclear.

Therefore, the aims of the study were to: (1) detect the probable ion channels or receptors involved in the mechanism by which Res relaxes the abdominal aorta from rats. (2) Give insights into the effect of Res on the extracellular calcium influx and intracellular calcium release.

2. Material and methods

2.1. Reagents

Resveratrol, N-G-nitro-L-arginine (L-NAME), tetraethylammonium (TEA), glybenclamide (Gly), atropine (AT), propranolol (PPL), indometacin (IMT), thapsigargin, nifedipine, sodium pentobarbital and L-norepinephrine bitartrate (NE) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals

Animal experiments were approved by the University Ethics Committee (This study conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health, NIH Publication No. 85-23, revised 1996). Prior to the experiments, male Sprague-Dawley (SD) rats (250–350 g body weight; 6–8 weeks old; from the animal center of the Fourth Military Medical University on Animal Care) had been reared at an ambient temperature of 23 ± 1 °C and a relative humidity of 60–70%, with light exposure daily from 6:00 to 18:00 h. Each animal received a standard laboratory diet and tap water ad libitum throughout the experimental period.

2.3. In vitro vascular perfusion

2.3.1. Vessel ring preparation

Rings, which are 2–3 mm in length, cut from the abdominal aorta obtained from the male SD rats (anesthetized with 40 mg/kg sodium pentobarbital intraperitoneally) were suspended for the measurement of their tension in organ chambers filled with Kreb's solution, maintained at 37 °C and bubbled with a gas mixture of 95% O_2–5% CO_2 (Warshaw et al., 1979). The Kreb's solution contained (in 10^{-3} mol/L) 118 NaCl, 4.8 KCl, 25 $NaHCO_3$, 2.5 $MgCl_2$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, and 11 glucose; pH 7.4. The tension transducer was prepared to record the tension of each vessel ring. After 1 h of equilibration with a basal tension of 1 g, norepinephrine (NE) was added into the chambers to assess the activity of each vessel ring. Kreb's solution in the chambers was renewed continuously till the tension descended to the baseline.

2.3.2. Endothelial function assessment

The endothelium of the vessel ring was removed by gently rubbing the intimal surface with a fine steel wire. The vessel ring constriction was triggered by 1 μmol/L NE till the tension went to its maximum, after which 1 μmol/L ACh was administrated to measure its dilation rate.

$$\text{dilation percentage} = \frac{\text{tension induced by the vasodilator}}{\text{tension induced by NE}} \times 100\%.$$

(The 100% dilation is defined by the vessel tension before NE is added) If the dilation rate is more than 80%, it indicates that the endothelium is still in good condition; if the dilation rate is less than 30%, it suggests that the integrity of the endothelium has been destroyed.

2.4. Statistical analysis

Data are expressed as the mean ± SEM. Student's *t*-test was used to determine the differences between two different groups. Statistical significance was determined at $p<0.05$ calculated by SPSS, version 10.0 (SPSS Science, Chicago, IL, USA).

3. Results

3.1. Vasorelaxing actions of Res in isolated abdominal aorta from rat

Concentration-dependent manner: When the tension of the vessel ring reached a steady state, 1 μmol/L NE was added to trigger vessel constriction (Fig. 1A). As the constriction arrived at its maximum and maintained that level, Res was given in a cumulatively increasing concentration manner. The cumulative concentration-relaxation curve indicated that Res began to dilate the vessel at 10^{-8} mol/L, and the vessel achieved 100%, the maximum dilation rate, at 10^{-4} mol/L ($n=6$) (Fig. 1B and C).

Time-dependent manner: Res at the dose of 5×10^{-5} mol/L suggested an obviously dilating effect on the vessel ring, so Res of that concentration was applied to explore the time-dependence of vasodilation. The time-relaxation curve indicated that after Res had been added, the ring began to dilate at 30 s and achieved the maximum of 100% dilation rate at 20 min ($n=6$) (Fig. 1D).

During the research into the time-dependent manner of Res, we noticed that the vessel ring might achieve its maximal dilation rate of 100% at the concentration of 50 μmol/L, we therefore considered that a relatively precise dose (concentration) would be found at the range of 10–100 μmol/L. When adding 10, 20, 30, 40, and 50 μmol/L Res to the chamber, we discovered that at the concentration of 40 μmol/L, the ring achieved its maximal dilation rate of 100% (Fig. 1E).

3.2. The effects of several different blockers on the vasorelaxing actions of Res

L-NAME (eNOS inhibitor): We added L-NAME (300 μmol/L) to the chamber. After 10 min, the vessel ring was triggered by 1 μmol/L NE to achieve the maximal constriction level. While the constriction level had remained stable for several minutes, 40 μmol/L Res was administered. The vessel dilation effect was reduced by (79.8 ± 3.7)%, ($n=6, p<0.05$) compared with the control group (Fig. 2B).

Gly (K^+_{ATP} blocker): We added 10 μmol/L Gly to the chamber. After 10 min, the vessel ring was triggered by 1 μmol/L NE to achieve the maximal constriction level. While the constriction level had remained stable for several minutes, 40 μmol/L Res was added. The vessel dilation effect was reduced by (43.8 ± 2.8)%, ($n=6, p<0.05$) compared with the control group (Fig. 2C).

TEA (BK_{Ca} blocker): We added 10 μmol/L TEA to the chamber. After 10 min, the vessel ring was triggered by 1 μmol/L NE to achieve the maximal constriction level. While the constriction level had remained stable for several minutes, 40 μmol/L Res was added. The vessel dilation effect was reduced by (38.2 ± 5.3)%, ($n=6, p<0.05$) compared with the control group (Fig. 2D).

AT (muscarinic receptor blocker), PPL (non-selective beta blocker) and IMT (cyclooxygenase inhibitor): We added 1 μmol/L AT, 5 μmol/L PPL and 10 μmol/L IMT respectively to the chamber. After 10 min, the vessel ring was triggered by 1 μmol/L NE to achieve the maximal constriction level. While the constriction level had remained stable for several minutes, 40 μmol/L Res was added. The maximal relaxing effect of abdominal aorta was not altered significantly compared with the control group ($n=6, p>0.05$) (Fig. 2E, F and G).