Resveratrol, a Component of Red Wine, Elicits Dilation of Isolated Porcine Retinal Arterioles: Role of Nitric Oxide and Potassium Channels

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PURPOSE. Resveratrol, a polyphenolic phytoalexin found in grapes and red wine, has been shown to exert cardiovascular benefits, but its action in the retinal microcirculation remains unknown. In this study, the direct effect and the underlying mechanism of the vasomotor action of resveratrol were examined in retinal arterioles.

METHODS. Porcine retinal arterioles were isolated, cannulated, and pressurized without flow for in vitro study. Resveratrol-induced diameter changes were recorded by videomicroscopic techniques.

RESULTS. Retinal arterioles (65 ± 3 μm) dilated dose dependently in response to resveratrol (1–50 μM). The removal of the endothelium reduced this dilation by 50%. Inhibition of nitric oxide (NO) synthase (by L-NAME; Nω-nitro-arginine methyl ester) and blockade of soluble guanylyl cyclase (by ODQ; 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one) produced similar inhibition as that produced by denudation. However, the resveratrol response was not affected by indomethacin (a cyclooxygenase inhibitor) and sulfaphenazole (an epoxygenase inhibitor). Intraluminal administration of an extracellular signal-regulated kinase (ERK) inhibitor (PD98059), but not an ase inhibitor. Intraluminal administration of an extracellular signal-regulated kinase (ERK) inhibitor (PD98059), but not an endothelium-dependent relaxation, activate nitric oxide (NO) synthase (NOS), inhibit platelet aggregation, and prevent oxidation of low-density lipoprotein cholesterol.3–5 Resveratrol (trans-3,4’,5-trihydroxyestilbene) is a polyphenolic phytoalexin, an antioxidant with known antifungal properties produced by several plants, and is found abundantly in the skin of red grapes and as a component of red wine. Since resveratrol has effects similar to those induced by RWPCs, especially in the promotion of vasorelaxation,6,7 its presence wine is thought to be responsible for red wine’s beneficial cardiovascular effects.

Epidemiologic studies indicate a close association between moderate consumption of red wine and reduced coronary heart disease and cardiovascular-related mortality.1,2 It has also been shown that red wine polyphenolic compounds (RWPCs) promote endothelium-dependent relaxation, activate nitric oxide (NO) synthase (NOS), inhibit platelet aggregation, and prevent oxidation of low-density lipoprotein cholesterol.3–5 Resveratrol has effects similar to those induced by RWPCs, especially in the promotion of vasorelaxation,6,7 its presence wine is thought to be responsible for red wine’s beneficial cardiovascular effects.

Because resveratrol can cause vascular relaxation6 and inhibit angiogenesis,15,16 it merits further investigation as a novel therapeutic agent for ocular vascular disorders, especially diabetic retinopathy, which is associated with impaired ocular circulation.15,16 However, there has been no study available to date that has been focused on the effect of resveratrol on ocular circulation. Using isolated vessel approaches, we examined the direct effect of resveratrol on retinal microvascular diameter and investigated the signaling mechanisms involved in this vasomotor activity.

MATERIALS AND METHODS

Animal Preparation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Scott & White Institutional Animal Care and Use Committee. Pigs (8–12 weeks old of either sex; 7–10 kg) purchased from Barfield Farms (Rogers, TX) were sedated with tiletamine-zolazepam (4.4 mg/kg, intramuscularly [IM], Telazol; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (2.2 mg/kg, IM), anesthetized with pentobarbital sodium (30 mg/kg, IV), intubated, and ventilated with room air. Heparin (1000 U/kg) was injected into the marginal ear vein to prevent clotting, and the eyes were enucleated and immediately placed in a moist chamber on ice.

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Isolation and Cannulation of Microvessels

The techniques for identification and isolation of retinal microvessels have been described. In brief, the anterior segment and vitreous body were removed carefully, guided by a dissection microscope. The posterior segment, or eye cup, was placed in a cooled dissection chamber (−8°C) containing a physiological salt solution (PSS [in mM]: NaCl 145.0, KCl 4.7, CaCl2 2.0, MgSO4 1.17, NaH2PO4 1.2, glucose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS [3-(N-morpholino)propanesulfonic acid] 3.0) with 1% albumin (USB, Cleveland, OH). Single second-order retinal arterioles (internal diameter in situ, 90–130 μm; 0.6–1.0 mm in length) were carefully dissected with a pair of Dumont microdissection forceps (Fine Science Tools, Foster City, CA) with the aid of a stereomicroscope (model SZX12; Olympus, Melville, NY). After careful removal of any remaining neural-connective tissues, the arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution equilibrated with room air at ambient temperature. One end of the arteriole was cannulated with a glass micropipette (tip outer diameter, 30–40 μm) filled with PSS-albumin solution, and the outside of the arteriole was securely tied to the pipette with an 11-0 ophthalmic suture (Alcon, Fort Worth, TX). The other end of the vessel was cannulated with a second micropipette and also secured with a suture. After cannulation, the vessel and pipettes were transferred to the stage of an inverted microscope (model CKX41; Olympus) coupled to a video camera (Sony DXC-190; Labtek, Campbell, CA), a video micrometer (Cardiovascular Research Institute, Texas A&M Health Science Center, College Station, TX), and a data-acquisition system (PowerLab; ADInstruments, Colorado Springs, CO) for continuous measurement and recording of the internal diameter throughout the experiment.19 The micropipettes were connected to independent pressure reservoirs (i.e., 30-ml glass syringes with 10-ml PSS). By adjusting the height of the reservoirs, we pressurized the vessel to 55 cmH2O (40–40 mm Hg) intraluminal pressure without flow. This level of pressure was used based on pressure ranges that have been documented in retinal arterioles in vivo and in the isolated, perfused retinal microcirculation and is consistent with the estimated ocular perfusion pressure in humans, as reported previously.20 Pressure in the vessel was kept constant throughout the experiment.21 Preparations with side branches and leaks were excluded from further study.

Experimental Protocols

Cannulated arterioles were bathed in PSS at 36°C to 37°C, to allow development of basal tone. After a stable basal tone developed (30–40 minutes), the dose-dependent vasodilation induced in the vessels by resveratrol (1–50 μM) was recorded. After the control dose-dependent response was measured, the vessels were washed with PSS to allow the redevelopment of basal tone and, in some studies, the vasodilation elicited by resveratrol was re-examined after 30 minutes, to confirm the reproducibility of the response. The vessels were exposed to each concentration of resveratrol for 3 to 5 minutes, until a stable diameter was established.

To elucidate the signaling mechanisms involved in the retinal arteriolar dilation induced by resveratrol, the following series of experiments were performed. The role of endothelium in the resveratrol-induced dilation was evaluated by comparing the response before and after removal of the endothelium. The vessel was perfused with a nonionic detergent, 3-(3-cholamidopropyl)dimethylammonio)1-propane sulfonate (CHAPS, 0.4%), for 1 to 2 minutes, to remove endothelial cells.22,23 To ensure that the vascular smooth muscle function was not compromised by the CHAPS treatment, dose-dependent dilation of the vessel in response to the endothelium-independent vasodilator sodium nitroprusside (0.1–100 μM) was examined before and after denudation. Only vessels that exhibited normal basal tone showed no vasodilation in response to endothelium-dependent vasodilator bradykinin (10 nM),24 and that showed unaltered vasodilation in response to sodium nitroprusside after removal of the endothelium were accepted for further study with resveratrol. The involvement of endothelium-derived vasodilators (i.e., prostaglandins, NO, and cytochrome P450 metabolites) in mediating the vascular response was assessed in the presence of known effective concentrations of the specific enzyme inhibitors indomethacin (10 μM),20,27 N6-nitro-L-arginine methyl ester (L-NAME; 10 μM),19,28 and sulfaphenazole (10 μM),29 respectively. The role of guanylyl cyclase/cGMP signaling was assessed by treating the vessels with the soluble guanylyl cyclase inhibitor 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 0.1 μM),20,21 in the absence or presence of the cGMP phosphodiesterase inhibitor zaprinast (1 μM).20 To probe the involvement of extracellular signal-regulated kinase (ERK), we studied the resveratrol-induced response after incubating the vessels with the ERK inhibitor PD98059 (1 μM).97 Since activation of potassium channels is known to be a major mechanism in vasodilation,30 we examined this pathway by treating the vessels with various potassium channel inhibitors: the nonselective potassium channel blocker tetraethylammonium (TEA, 20 mM), the large-conductance Ca2+-activated potassium (BKCa) channel blockeriberiotoxin (0.1 μM),31,32 the voltage-gated K+ (Kv) channel inhibitor 4-aminopyridine (4-AP, 0.1 mM),33 or the ATP-sensitive potassium (KATP) channel blocker glibenclamide (5 μM).20 TEA has been reported to inhibit BKCa channels selectively at concentrations lower than 1 mM, while blocking other potassium channels at higher concentrations (i.e., up to 10 mM).31 Because resveratrol is structurally similar to the synthetic estrogen diethylstilbestrol,34 we examined the role of estrogen receptors by Resveratrol-induced dilation of retinal arterioles. The involvement of estrogen receptor antagonist ICI 182780 (1 μM) for 30 minutes before we examined the resveratrol-induced response.55 Sodium nitroprusside (1 mM to 10 mM) was used to probe endothelium-independent NO-mediated vasodilation. All drugs were administered extraluminally, unless otherwise stated. Each pharmacologic inhibitor was incubated with the vessels for at least 30 minutes.

Chemicals

Drugs were obtained from Sigma-Aldrich (St. Louis, MO). L-NAME, TEA, iberiotoxin, 4-AP, and sodium nitroprusside were dissolved in PSS. Indomethacin, sulfaphenazole, and ODQ were dissolved in ethanol. Resveratrol, PD98059, ICI 182780, and glibenclamide were dissolved in dimethyl sulfoxide (DMSO). Subsequent dilutions of these drugs were prepared in PSS. The final concentration of ethanol and DMSO in the vessel bath was less than 0.1%. Vehicle control studies indicated that this final concentration of solvent had no effect on the arteriolar function.

Data Analysis

At the end of each experiment, the vessel was relaxed in EDTA (1 mM) calcium-free PSS, to obtain its maximum diameter at 55 cmH2O intraluminal pressure.19,21 All diameter changes in response to agonists were normalized to this maximum vasodilation and expressed as a percentage of maximum dilation.19,21 Data are reported as the mean ± SEM, and n represents the number of vessels studied. Statistical comparisons of the change in resting tone by antagonists were performed by Student’s t-test. Two-way ANOVA, followed by the Bonferroni multiple-range test, was used to determine the significance of the difference between control and experimental interventions. P < 0.05 was considered significant.

RESULTS

Dilation of Retinal Arterioles Induced by Resveratrol

In this study, all vessels (n = 60) had basal tone in the range of 50% to 70% (average, ~64% ± 2%) of their maximum diameter in a 36°C to 37°C bath temperature with 55 cmH2O intraluminal pressure. The average resting and maximum diameters of the vessels were 65 ± 3 and 101 ± 2 μm, respectively. Resveratrol produced a consistent dose-dependent dilation of
retinal arterioles, and the vasodilatory response reached its maximum within 2 to 3 minutes. The threshold concentration for vasodilation was 3 μM and the highest concentration (50 μM) elicited approximately 60% of maximum dilation (Fig. 1). To avoid the confounding effects from the high concentration of solvent (i.e., DMSO), concentrations of resveratrol higher than 50 μM were not examined. Further study showed that resveratrol-induced dilation was reproducible and did not deteriorate after repeated application (Fig. 1).

Role of Endothelium

In this series of studies, 10 vessels were subjected to the denudation protocol. After perfusion with CHAPS, 3 of 10 vessels lost basal tone and 3 showed partial inhibition by the endothelium-dependent vasodilator bradykinin. These apparently damaged or partially denuded vessels were excluded from further study. The remaining four vessels maintained basal tone (control: 65% ± 5% vs. denudation: 67% ± 6%; P = 0.87) and the vasodilation induced by bradykinin (10 nM) was abolished (control: 91% ± 5% vs. denudation: 1% ± 1%). In addition, these vessels exhibited normal vasodilation in response to sodium nitroprusside (Table 1). In these accepted denuded vessels, the dilation induced by resveratrol was partially reduced and the response to the highest concentration of resveratrol was reduced from 54% to 26% (P < 0.001; Fig. 2).

Role of Endothelium-Derived Factors

The relative contribution of NO, cyclooxygenase-derived prostaglandins, and cytochrome P450 metabolites to resveratrol-induced vasodilation was assessed by their respective inhibitors. Inhibition of cytochrome P450 epoxygenase and prostaglandins by sulfaphenazole and indomethacin, respectively, did not affect the vasodilatory response to resveratrol (Fig. 3). The NOS inhibitor L-NAME exhibited an inhibitory effect on resveratrol-induced vasodilation (P < 0.001) comparable to that produced by the denudation (L-NAME versus denudation; P > 0.05; Figs. 2, 3). The basal tone was not significantly altered by sulfaphenazole (control: 66% ± 4% vs. sulfaphenazole: 68% ± 1%; P = 0.18), indomethacin (control: 69% ± 1% vs. indomethacin: 67% ± 1%; P = 0.49), and L-NAME (control: 71% ± 2% vs. L-NAME: 68% ± 3%; P = 0.57).

Role of Guanylyl Cyclase

To assess whether the activation of soluble guanylyl cyclase mediates resveratrol-induced vasodilation, the retinal arterioles were treated with its inhibitor ODQ for 30 minutes. As shown in Figure 4, ODQ significantly reduced the vasodilatory response to resveratrol in a manner similar to L-NAME. Basal tone was slightly increased by ODQ (control: 72% ± 3% vs. ODQ: 67% ± 2%; P = 0.04). The inhibitory effect of ODQ was not affected by treating the vessels with the cGMP phosphodiesterase inhibitor zaprinast, indicating that the increased cGMP

![Figure 1](image1.png) **Figure 1.** The response of isolated retinal arterioles to resveratrol. The dose-dependent vasodilatory effect of resveratrol was examined (first trial; resting diameter: 71 ± 7 μm; maximum diameter: 105 ± 6 μm; n = 7) and then repeated after a 30-minute washout period (second trial; resting diameter: 71 ± 7 μm; maximum diameter: 105 ± 6 μm; n = 7).

![Figure 2](image2.png) **Figure 2.** The role of endothelium in the retinal arteriolar dilation in response to resveratrol. The dose-dependent vasodilatory effect of resveratrol was examined before (control; n = 4) and after removal of the endothelium (n = 4) by perfusion with 0.4% CHAPS. *P < 0.05 versus control.

### Table 1. Diameter Responses of Retinal Arterioles to Sodium Nitroprusside

<table>
<thead>
<tr>
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<th>Sodium Nitroprusside (μM)</th>
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<tr>
<td></td>
<td>0.1</td>
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<tr>
<td>Control</td>
<td>4.6 ± 1.5</td>
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<tr>
<td>Denudation</td>
<td>3.2 ± 3.2</td>
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<tr>
<td>L-NAME</td>
<td>2.5 ± 1.6</td>
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<tr>
<td>PD98059</td>
<td>4.9 ± 1.6</td>
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<tr>
<td>TEA</td>
<td>3.4 ± 1.9</td>
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<tr>
<td>Iberiotoxin</td>
<td>1.7 ± 1.7</td>
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<tr>
<td>L-NAME + Iberiotoxin</td>
<td>4.7 ± 1.0</td>
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Data are expressed as the mean ± SEM. n, number of vessels. Based on two-way ANOVA, responses to sodium nitroprusside were not affected by any perturbations, compared with control.
from soluble guanylyl cyclase, rather than from other sources (e.g., particulate isofrom), was responsible for the retinal arteriolar dilation induced by resveratrol.

Role of ERK and Estrogen Receptors
To assess the role of ERK and estrogen receptors in the endothelium in mediating resveratrol-induced vasodilation, we treated the retinal arterioles intraluminally with specific blockers PD98059 (ERK) and ICI 182780 (estrogen). Intraluminal administration of PD98059 or ICI 182780 did not affect basal tone of retinal arterioles but only PD98059 significantly inhibited the vasodilation in response to resveratrol (Fig. 5).

Role of Potassium Channels
TEA significantly reduced resveratrol-induced dilation of retinal arterioles (Fig. 6), but glibenclamide and 4-AP were ineffective. In addition, administration of iberiotoxin (0.1 μM) reduced dilation in the same manner as did the application of TEA. The residual vasodilation in the presence of extraluminal iberiotoxin was further reduced by subsequent treatment with L-NAME (Fig. 6). The basal tone was not significantly altered by TEA (control: 59% ± 5% vs. TEA: 57% ± 5%; P = 0.26), iberiotoxin (control: 59% ± 5% vs. iberiotoxin: 60% ± 4%; P = 0.52), or the combination of iberiotoxin and L-NAME (control: 53% ± 6% vs. iberiotoxin + L-NAME: 52% ± 5%; P = 0.50).

Response to Sodium Nitroprusside
Table 1 shows the effects of various interventions on the dilation of retinal arterioles in response to sodium nitroprusside. The sodium nitroprusside–induced dilations were not affected by L-NAME, PD98059, TEA, iberiotoxin, or combined L-NAME and iberiotoxin, indicating that vascular smooth muscle function was not altered by these pharmacologic interventions.

DISCUSSION
In the present study, we demonstrated for the first time that resveratrol induces dose-dependent dilation of small retinal arterioles in response to resveratrol. Dose-dependent vasodilation induced by resveratrol was examined before (control; n = 17) and after incubation with the NOS inhibitor L-NAME (10 μM; n = 7), the cyclooxygenase inhibitor indomethacin (10 μM; n = 7), or the cytochrome P450 epoxygenase inhibitor sulfaphenazole (10 μM; n = 3). *P < 0.05 versus control.

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932943/ on 06/24/2017)

**FIGURE 3.** The role of endothelium-derived factors in the dilation of retinal arterioles in response to resveratrol. Dose-dependent vasodilation induced by resveratrol was examined before (control; n = 17) and after incubation with the NOS inhibitor L-NAME (10 μM; n = 7), the cyclooxygenase inhibitor indomethacin (10 μM; n = 7), or the cytochrome P450 epoxygenase inhibitor sulfaphenazole (10 μM; n = 3). *P < 0.05 versus control.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932943/ on 06/24/2017)

**FIGURE 4.** The role of guanylyl cyclase in the vasodilatory response to resveratrol of the isolated retinal arteriole. The dose-dependent vasodilatory response to resveratrol was examined before (control; n = 9) and after incubation with the soluble guanylyl cyclase inhibitor ODQ (0.1 μM; n = 9) in the absence or presence of the cGMP phosphodiesterase inhibitor zaprinast (1 μM; n = 4). *P < 0.05 versus control.

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932943/ on 06/24/2017)

**FIGURE 5.** The role of the ERK/MAP kinase pathway and estrogen receptor in the vasodilatory response to resveratrol of the endothelium of the isolated retinal arteriole. The dose-dependent vasodilatory response to resveratrol was examined before (control; n = 10) and after intraluminal incubation with the ERK inhibitor PD98059 (1 μM; n = 7) or the estrogen receptor antagonist ICI 182780 (1 μM; n = 3). *P < 0.05 versus control.
Figure 6. The role of potassium channels in the vasodilatory response to resveratrol of the isolated retinal arteriole. Dose-dependent vasodilation in response to resveratrol was examined before (control; n = 17) and after the extraluminal incubation with TEA (20 mM; n = 5), glibenclamide (5 μM, n = 3), 4-AP (0.1 mM; n = 4), and ibetox (0.1 μM; n = 5). Residual vasodilation in the presence of ibetox (0.1 μM) was examined after incubation with t-NAME (10 μM; n = 4). IbTx, ibetox. *P < 0.05 versus control. **P < 0.05 IbTx versus IbTx + t-NAME.

arterioles. This vasodilatory response was repeatable, and no apparent tachyphylaxis was observed. Although previous studies in the aorta,10,50 and conduit arteries57–59 in various vascular beds other than the retina have demonstrated the vasodilatory effect of resveratrol in vitro, we extend these findings to the downstream small-resistance arterioles (~70 μm internal diameter). This observation has physiologic and pathophysiologic implications, because blood flow is functionally regulated by these small-sized arterioles.64

The present study demonstrated that resveratrol induces dose-dependent dilation of retinal arterioles with threshold concentration at 3 μM (Fig. 1). Experimental studies have shown that after oral administration of resveratrol at dietary-relevant doses, significantly higher concentrations of resveratrol accumulate in tissues such as the heart, liver, and kidney (~10–30 μM) than in plasma (0.1–1.5 μM).35,46 Because of its lipophilic nature, the tissue level of resveratrol may provide a better indicator of the vasculature’s exposure to biologically active concentrations. Bertelli et al.67 suggested that an average drinker of red wine can, particularly in the long term, absorb a sufficient quantity of resveratrol in the tissue to exert its cardiovascular benefits. Although the level of resveratrol in ocular tissue has not been measured, it is likely that regular and moderate consumption of red wine and/or natural polyphenolic compounds would enable retinal tissue to maintain the required resveratrol level for vasomotor activation. Taking all evidence together, it is reasonable to consider that resveratrol has a therapeutic potential in retinal vascular disease.

Our data show that endothelial disruption reduced, but did not abolish, the vasodilation induced by resveratrol (Fig. 2), suggesting that resveratrol exerts both endothelium-dependent and -independent effects on retinal arterioles. Although previous studies have examined the involvement of endothelium in the resveratrol-induced response, the effects of denudation on resveratrol-induced dilation have been inconsistently reported. Some studies showed a partial (i.e., 20%–30% in sheep40 and porcine coronary arteries and 40% and 80% in human saphenous vein and internal mammary artery, respectively59) or complete (i.e., rat aortic rings58) endothelial dependency, whereas a shifting from partial endothelium-dependent to complete endothelium-independent was reported in rat mesenteric arteries after dietary-induced obesity48 and in small peripheral arteries in humans with established coronary heart disease.42 It appears that the role of endothelium in resveratrol-induced dilation depends on the tissue, species, and perhaps disease states. In our studies, the endothelium contributed to the dilation of retinal arterioles induced by resveratrol by approximately 50% (Fig. 2). The involvement of both endothelium-dependent and -independent components seems to be desirable for clinical use of resveratrol, because the endothelium-independent component of resveratrol-induced vasodilation can become more important under some clinical conditions,57 especially in association with compromised endothelial function, such as dietary obesity, type II diabetes, hypertension, and hypercholesterolemia.

In the present study, we found that blockade of NOS partially inhibited the resveratrol-induced response, mimicking the effect of denudation and suggesting that NO contributes entirely to the endothelium-dependent component of vasodilation in response to resveratrol (Fig. 3). This contention is further supported by the observations that the resveratrol response was unaffected by inhibition of synthesis of prostanoid and cytochrome P450 metabolites, the other two important vasodilators released from the endothelium. The involvement of NO in resveratrol-induced vasodilation was also reported in various studies in different species and vascular beds.8,56–58,60–65,45,48 However, there has been no other study to examine whether cytochrome P450 metabolites are involved in the vasodilatory response to resveratrol. In contrast, somewhat disparate results have been reported regarding the role of indomethacin-sensitive prostanoids. Although enzyme kinetic studies of cyclooxygenase proteins in a cell-free system have shown that resveratrol selectively inhibits activity of the cyclooxygenase-1 isozyme,59,60 it is possible that stimulation of other signaling pathways by resveratrol due to its pleiotropic effects51 indirectly activates cyclooxygenase or specific prostaglandin synthases and subsequently influences the vasomotor response in intact blood vessels. Indeed, previous studies have shown that indomethacin can slightly increase the resveratrol-induced relaxation of human internal mammary arteries45 and guinea pig uterine arteries,11 implicating a potential reduction of vasoconstrictor prostanoids. However, the inability of indomethacin to alter the resveratrol-induced dilation of retinal arterioles in our study suggests minimal, if any, involvement of cyclooxygenase-1-derived prostanoids in modulating the vascular response to resveratrol. This result is consistent with the negative findings in guinea pig41 and rat48 mesenteric arteries, as well as porcine coronary arteries.45 It is likely that the influence of vasoconstrictor prostanoids on vascular reactivity in response to resveratrol is tissue dependent. All evidence taken together, the endothelium-dependent component of resveratrol-induced dilation in retinal arterioles is mainly contributed by NO, independent of prostanoids and cytochrome P450 metabolites.

In our experiment, the time course of the vasomotor response indicates that resveratrol elicits retinal arteriolar dilation within a few minutes. Recent studies have shown that short-term exposure to resveratrol (for 2 minutes) can enhance the release of NO from cultured endothelial cells.7 In addition, resveratrol (10 μM) can increase cGMP formation by 2.5 times within 10 minutes in sheep coronary arteries.40 We speculate
that the molecular mechanism behind this rapid effect can be related to a prompt enhancement of endothelial (e)NOS activity. Of interest, recent studies have reported that the ERK signaling cascade is involved in the rapid (<5 minute) activation of eNOS for NO production and that resveratrol (50 nM) can rapidly activate MAP kinase and eNOS in cultured endothelial cells. Moreover, resveratrol was recently shown to trigger a preconditioning-like survival pathway by activating MAP kinase signaling cascade involving ERK in the isolated rat heart. In the present study, inhibition of the ERK pathway reduced resveratrol-induced dilation of retinal arteries in a same manner as dila-L-NAME and denudation. It appears that activation of the ERK pathway is involved in the cGMP-mediated dilation of retinal arteries in response to resveratrol, probably via the rapid activation of eNOS.

Of interest, resveratrol has structural similarity to a synthetic estrogen diethylstilbestrol and has been shown to exhibit estrogen receptor agonism in various cell culture models. It is possible that estrogen receptor activation contributes to the retinal arteriolar dilation in response to resveratrol since a recent study demonstrated that this phytoestrogen compound rapidly activates MAP kinase signaling for eNOS activation through estrogen receptors in cultured endothelial cells. However, in the present study, we did not observe any changes in resveratrol-induced vasodilation by the selective estrogen receptor blocker ICN 182780. This negative result is consistent with the ineffectiveness of estrogen receptor antagonists in blocking resveratrol-induced relaxation in porcine coronary artery. It is worth noting that the concentration of ICN 182780 (1 μM) used in the present study effectively blocked the dilation (control: 69 ± 1% vs. ICN 182780: 9% ± 3%) of isolated porcine coronary arteries in response to 17-β-estradiol (5 μM, n = 3). In contrast to coronary arteries, the retinal arteries responded little to 17-β-estradiol (5 μM, 11% ± 1% dilation, n = 3), indicating the sparse estrogen receptor distribution and/or the insensitivity of estrogen receptors in retinal arteries. Although the reason underlying the inconsistent result on the role of estrogen receptors in resveratrol-induced responses remains unclear, it may be related to tissue specificity, intact tissue versus cell culture, and/or the conditions of experimental settings.

In the present study, resveratrol-induced vasodilation was partially inhibited by ODQ (Fig. 4) in a manner identical with that produced by denudation (Fig. 2) and L-NAME (Fig. 3), suggesting the involvement of the soluble guanyl cyclase/cGMP pathway in the endothelium-dependent component of vasodilation in response to resveratrol. This contention is supported by findings in sheep coronary arteries showing that resveratrol-induced cGMP production can be attenuated by denudation, NOS inhibitor, or ODQ. However, the latter study also reported that resveratrol-stimulated cGMP production from the particulate membrane fraction of smooth muscle cells. Moreover, the resveratrol-induced cGMP production in denuded sheep coronary artery was further enhanced by zaprinast, a phosphodiesterase inhibitor that selectively inhibits cGMP degradation. It appears that resveratrol can also activate cGMP synthesis from particulate guanylyl cyclase independent of endothelium. With this consideration, if particulate guanylyl cyclase had contributed to retinal arteriolar dilation, we would have seen the vasodilatory response to resveratrol enhanced by zaprinast, under the condition of blockade of soluble guanylyl cyclase activity. However, in the presence of ODQ, we found that the resveratrol-induced dilation was not enhanced by zaprinast (Fig. 4) at a concentration sufficient to increase cGMP-mediated dilation in isolated retinal arteries. Therefore, it is unlikely that the activation of the particulate guanylyl cyclase/cGMP pathway is involved in the dilation of retinal arterioles induced by resveratrol in the present study.

Our data show that denudation reduced the resveratrol-induced response by only 50% (Fig. 2), clearly indicating that there is an endothelium-independent component of resveratrol-induced dilation in retinal arteries. Since potassium channels play a cardinal role in the regulation of smooth muscle tone in the peripheral microcirculation, we examined whether these channels contribute to the endothelium-independent effect of resveratrol. We found that resveratrol-induced dilation was significantly inhibited by the nonselective potassium channel blocker TEA, suggesting the involvement of potassium channels in resveratrol-induced vasodilation in retinal arteries. It has been reported that the activation of KATp channels is responsible for the dilation of porcine retinal arterioles induced by adenosine54,55 and lactate. In contrast, KCa channels are involved in the calcitonin gene-related peptide-induced dilation of bovine retinal arteries, since 4-AP selectively attenuates this response. However, it is unlikely that these two types of potassium channels are involved in the resveratrol-induced response of retinal arteries, because their respective inhibitors, glibenclamide and 4-AP, had no effect on the vasodilatory response in the present study (Fig. 6). In contrast, the BKCa channel blocker iberiotoxin inhibited resveratrol-induced dilation in the same manner as a nonselective potassium channel blocker TEA (Fig. 5), suggesting that the BKCa channel is primarily responsible for the effect of resveratrol. Because NO is involved in the resveratrol-stimulated dilation of retinal arteries and several studies have reported that NO is capable of activating BKCa channels in some types of vascular57–60 it is possible that NO was linked to BKCa channel activation in the present study. However, we found that the vasodilation caused by the NO donor sodium nitroprusside was not affected by iberiotoxin (Table 1). In addition, coincubation of iberiotoxin with L-NAME in the intact vessel (Fig. 6) almost abolished the response to resveratrol, suggesting that the release of endothelial NO and the BKCa activation in smooth muscle are independent pathways for resveratrol-induced vasodilation.

In summary, the present study demonstrated that resveratrol elicits a marked dilation of retinal arteries. This dilation consists of both endothelium-dependent and -independent components. The endothelium-dependent response is mediated by the activation of eNOS, probably through ERK/MAP kinase signaling, for NO release and the consequent activation of soluble guanylyl cyclase/cGMP pathway. The endothelium-independent component is mainly mediated by the activation of BKCa channels in the smooth muscle. Understanding the mechanisms involved in the resveratrol-stimulated vasodilation of retinal arteries could be therapeutically valuable for ocular vascular disorders. Clearly, further clinical study is needed to examine the effect of resveratrol on the ocular circulation in patients with ocular vascular disease.

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