Simvastatin Elicits Dilation of Isolated Porcine Retinal Arterioles: Role of Nitric Oxide and Mevalonate-Rho Kinase Pathways

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PURPOSE. Results in a prior study have demonstrated that systemic administration of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor simvastatin to healthy subjects reduces intraocular pressure and increases retinal blood flow. However, it remains unclear whether simvastatin can directly elicit dilation of retinal microvessels. In the current study, the direct effect and the underlying mechanism of the vasomotor action of simvastatin in retinal arterioles was studied.

METHODS. Porcine retinal arterioles (~75 μm internal diameter) were isolated, cannulated, and pressurized (55 cmH2O) without flow for in vitro study. Diameter changes in response to simvastatin were recorded using videomicroscopic techniques.

RESULTS. Retinal arterioles dilated dose dependently to simvastatin (1 nM to 10 μM). This vasodilation was significantly reduced after removal of the endothelium. The nitric oxide (NO) synthase inhibitor l-NAME (Nω-nitro-l-arginine methyl ester) markedly inhibited the vasodilation, and combined administration of l-NAME with cyclooxygenase inhibitor indomethacin mimicked the effect of demedulation. Blockade of soluble guanylyl cyclase by ODQ (1H-[1,2,4] oxadiazolo[4,3,-alquinoxaline-1-one) produced a similar inhibitory effect as that by l-NAME. In contrast, the dilation was unaffected by cytochrome-P450 epoxidegenase inhibitor sulfaphenazole. Intraluminal incubation of vessels with mevalonate, an immediate metabolite of HMG-CoA reductase, partially inhibited vasodilation to simvastatin. The Rho kinase inhibitor Y-27632 abolished the antagonistic effect of mevalonate.

CONCLUSIONS. Simvastatin elicits mainly an endothelium-dependent, NO-mediated dilation of retinal arterioles via activation of guanylyl cyclase; cyclooxygenase plays a relatively minor role. It appears that inhibition of the mevalonate-Rho kinase pathway in endothelial cells contributes in part to the simvastatin-induced vasodilation. A better understanding of the action of statins on retinal vasculature may help shed light on its therapeutic potential in retinal vascular disease. (Invest Ophthalmol Vis Sci. 2007;48:825–832) DOI:10.1167/iovs.06-0856

In addition to the inhibition of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (i.e., statins) have been shown to improve endothelium-dependent relaxation1 and possibly to reduce cardiovascular risk, even in patients with normal cholesterol levels.2,3 These findings suggest that statins may exert pleiotropic effects with vascular protection beyond cholesterol reduction. Studies of the retinal tissue subjected to ischemia-reperfusion4 or of animals with streptozotocin-induced diabetes5 have suggested that simvastatin may exert a neuroprotective effect by inhibiting leukocyte-endothelial cell interaction through the release of nitric oxide (NO) from the endothelium. In addition, clinical studies have shown that long-term statin usage may contribute to risk reduction in various ocular disorders, such as age-related macular degeneration,6–8 diabetic retinopathy,9 and glaucoma.10 Because such ocular disorders can be associated with impaired ocular circulation,11–13 the direct role of statins in modulating ocular vasomotor function must be addressed, to provide further therapeutic insights into disease treatment.

Statins can be classified into three categories: naturally derived (lovastatin and pravastatin), semisynthetic (simvastatin), and synthetic (atorvastatin, fluvastatin, cerivastatin, rosuvastatin, and pitavastatin).14 It has been shown that statins that contain a lactone structure with high lipophilic properties—that is, simvastatin and lovastatin—exhibit potent vasodilatory effect in the intact heart15 and in vessels isolated from various tissues.16–20 However, it is not clear whether this vasomotor effect can also be found in the retinal arterioles. We recently looked into this question and demonstrated that systemic administration of simvastatin can increase blood flow in retinal arteries and veins in healthy human subjects21; but it is not clear whether this increased flow results from the dilation of the retinal microvessel, because a reduction of intraocular pressure was also found to be associated with flow elevation. In conjunction with this study, Bayerle-Eder et al.22 reported that administration of pravastatin, another form of statin, enhances the NO effect on pulsatile choroidal blood flow in patients with hypercholesterolemia. Collectively, these animal and clinical results suggest that statins may have beneficial effects on the ocular circulation by increasing ocular blood flow and improving vascular function. However, it is unclear whether statins can directly exert a vasodilatory effect on retinal microvessels, because cholesterol reduction itself can improve vascular function23,24 and because the secondary changes in perfusion pressure, intraocular pressure, blood flow, and tissue metabolism that potentially influence vasomotor activity cannot be excluded in in vivo models. Furthermore, there has been no study to date to investigate directly the acute action of statins on the retinal microvasculature. Herein, using isolated vessels, we examined the direct effect of simvastatin on retinal microvascular diameter and investigated the signaling mechanisms involved in this vasomotor activity.

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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 telazol (4.4 mg/kg, intramuscular) and xylazine (2.2 mg/kg, IM), anesthetized with pentobarbital sodium (30 mg/kg, intravenous), intubated, and ventilated with room air. Heparin (1000 U/kg) was administered into the marginal ear vein to prevent clotting, and the eyes were enucleated and immediately placed in a moist chamber on ice.

Isolation and Cannulation of Microvessels
The techniques for identification and isolation of retinal microvessels have been described previously.25,26 In brief, the anterior segment and vitreous body were removed carefully under a dissecting 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 (in the range of 90–130 μm in internal diameter) were 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 and 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 by using a glass micropipette (tip outer diameter, 30–40 μm) filled with PSS-albumin solution, and the outside of the arteriole was secured tied to the pipette with 11-0 ophthalmic sutures (Alcon, Fort Worth, TX). The other end of the vessel was cannulated with a second micropipette and also secured with sutures. After cannulation, the vessel and micropipettes were transferred to the stage of an inverted microscope (model CKX41; Olympus) coupled to a video camera (Sony DCC-190; Labtek, Campbell, CA), video micrometer (Cardiovascular Research Institute, Texas A&M System Health Science Center, College Station, TX), and data acquisition system (PowerLab; ADInstruments, Colorado Springs, CO) for continuous measurement and recording of the internal diameter throughout the experiment.25 The micropipettes were connected to independent pressure reservoirs (i.e., 30-mL glass syringes). By adjusting the height of the reservoirs, we pressurized the vessel to 55 cm H2O (intraluminal pressure without flow). This level of pressure was used based on pressure ranges that have been documented in retinal arterioles in vivo27 and in the isolated, perfused retinal microcirculation,28 and was consistent with the estimated ocular perfusion pressure in humans as reported previously.21 Because the size (2.5 cm in diameter) and volume (10-mL PSS) of the reservoir is overwhelmingly larger than the microvessel (approximately 80 μm in diameter, 1.0 mm in length, and 5 mL in luminal volume), the changes in vessel diameter, and thus in volume, would not cause a significant change in the height of the PSS in the reservoir. Therefore, pressure in the vessel can be kept constant throughout the experiment. 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 vessels developed a stable basal tone (~30–40 minutes), the dose-dependent vasodilation to simvastatin, 1 nM to 10 μM, was constructed based on the evidence that the plasma level of simvastatin can reach 10 nM to 1 μM in patients treated with therapeutic doses of simvastatin.29–31 After the control responses were completed, the vessels were washed with PSS to allow the redevelop-
multiple-range test was used to determine the significance of difference between control and experimental interventions. \( P < 0.05 \) was considered significant.

**RESULTS**

**Vasodilation of Retinal Arterioles to Simvastatin**

In this study, all vessels \((n = 52)\) showed a similar level of basal tone (constricted to 68% ± 1% of their maximal diameter) at 36°C to 37°C bath temperature with 55 cmH2O intraluminal pressure. The average resting and maximal diameters of the vessels were 74 ± 2 and 109 ± 2 μm, respectively. Figure 1A shows the representative time course of the response of simvastatin to retinal arterioles. Simvastatin (10 μM) produced a robust dilation of an isolated arteriole from the baseline diameter of 80 to 96 μm (Fig. 1A) within 3 minutes (Fig. 1A). The diameter gradually returned to the baseline level after the vessel bath was replaced with PSS. In general, simvastatin produced dose-dependent dilation of retinal arterioles, and the dilation to each concentration of simvastatin was completely developed within 2 to 3 minutes. The threshold concentration for vasodilation was 0.1 μM, and the highest concentration (10 μM) elicited approximately 50% of maximal dilation (Fig. 1B). To avoid the confounding effects from the high concentration of solvent (i.e., ethanol), a concentration of simvastatin higher than 10 μM was not examined. Further study showed that simvastatin-induced dilation was reproducible and did not deteriorate after repeated application (Fig. 1B).

**Role of Endothelium**

In this series of studies, 10 vessels were subjected to denudation protocol. After perfusion with CHAPS, 2 of 10 vessels lost basal tone, 2 did not exhibit normal response to the endothelium-independent vasodilator sodium nitroprusside, and 1 showed partial inhibition in response to the endothelium-dependent vasodilator bradykinin. These apparently damaged or partially denuded vessels were excluded from further study. The remaining five vessels maintained basal tone (control: 65% ± 4% vs. denudation: 62% ± 4%, \( P = 0.08 \)) and the vasodilation in response to bradykinin was abolished (control: 83% ± 8% vs. denudation: 1% ± 1%). In addition, these vessels exhibited normal vasodilation with sodium nitroprusside (Table 1). In these accepted denuded vessels, the dilation to the lower concentrations of simvastatin (<10 μM) was abolished, and the response to the highest concentration of simvastatin was reduced from 50% to 18% as shown in Figure 2.

**Role of Endothelium-Derived Factors**

The relative contribution of NO, cyclooxygenase-derived prostaglandins, and cytochrome P450-derived EDHF to simvastatin-induced vasodilation was assessed by their respective inhibitors. Inhibition of cytochrome-P450 epoxygenase by sulphasphenazole did not affect the vasodilation in response to simvastatin (Fig. 3). Indomethacin produced a partial inhibitory response by reducing the vasodilation from 55% to 38% at the highest concentration of simvastatin (\( P < 0.01 \), two-way ANOVA with Bonferroni post hoc test; Fig. 3). The NO synthase inhibitor L-NAME exhibited a stronger inhibitory effect on simvastatin-induced vasodilation than did indomethacin. The residual vasodilation with 10 μM simvastatin in the presence of L-NAME was further reduced by the subsequent treatment with indomethacin (\( P < 0.05 \), two-way ANOVA with Bonferroni post hoc test; Fig. 3). The inhibition produced by the combined L-NAME and indomethacin was comparable to that produced by the denudation (L-NAME+indomethacin versus denudation; \( P > 0.05 \), two-way ANOVA with Bonferroni post-hoc test; Figs. 2, 3). The basal tone was not significantly altered by sulphasphenazole (control: 66% ± 4% vs. sulphasphenazole: 68% ± 2%, \( P = 0.22 \)), indomethacin (control: 74% ± 3% vs. indomethacin: 72% ± 3%, \( P = 0.35 \)) or L-NAME (control: 68% ± 3% vs. L-NAME: 66% ± 3%, \( P = 0.08 \)), but was slightly increased by the combination of L-NAME and indomethacin (control: 65% ± 4% vs. L-NAME+indomethacin: 57% ± 5%, \( P = 0.01 \)).

**Role of Guanylyl Cyclase**

To assess the role of soluble guanylyl cyclase in mediating simvastatin-induced vasodilation, the retinal arterioles were...
treated with the inhibitor ODQ for 30 minutes. As shown in Figure 4, ODQ significantly reduced the vasodilation response to simvastatin in a manner similar to L-NAME. It should be noted that ODQ did not alter basal tone (control: 66% ± 2% vs. ODQ: 67% ± 3%; P = 0.64).

Role of Mevalonate and Rho/Rho Kinase Pathway

Intraluminal administration of mevalonate did not affect the basal tone of retinal arterioles (control: 72% ± 2% vs. mevalonate: 71% ± 2%; P = 0.80) but significantly inhibited the vasodilation in response to simvastatin (Fig. 5). On the other hand, the inhibitory effect of mevalonate was prevented by the coadministration of the Rho kinase inhibitor Y-27632 (Fig. 5). The basal tone of retinal arterioles was not altered by this inhibitor (control: 59% ± 3% vs. mevalonate+Y-27632: 62% ± 3%; P = 0.18).

Response to Sodium Nitroprusside

Table 1 shows the effects of various interventions on the dilation of retinal arterioles to sodium nitroprusside. The sodium nitroprusside-induced dilations were not affected by L-NAME, indomethacin, or intraluminal mevalonate, indicating vascular smooth muscle function was not altered by these pharmacological interventions.

DISCUSSION

We have demonstrated that systemic administration of simvastatin causes an increase in retinal blood flow of healthy human subjects.21 This phenomenon is associated with a reduction in intraocular pressure and low-density lipoprotein (LDL) cholesterol in the serum.21 Bayerle-Eder et al.22 also reported that the improved choroidal vascular response to NO synthase (NOS) inhibitor by pravastatin is correlated with the reduction of the plasma total cholesterol level in hypercholesterolemic patients. Although these studies suggest a general beneficial effect of statins in the retinal circulation, an unequivocal determination of the direct role of statins in vascular regulation cannot be

<table>
<thead>
<tr>
<th>Sodium Nitroprusside (μM)</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
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<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 1.9</td>
<td>27.1 ± 5.1</td>
<td>60.3 ± 3.5</td>
<td>80.0 ± 5.9</td>
</tr>
<tr>
<td>Denudation</td>
<td>9.2 ± 4.3</td>
<td>35.1 ± 7.5</td>
<td>60.8 ± 5.8</td>
<td>78.7 ± 4.7</td>
</tr>
<tr>
<td>L-NAME</td>
<td>4.0 ± 1.7</td>
<td>24.9 ± 4.1</td>
<td>64.8 ± 6.5</td>
<td>83.0 ± 7.7</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.7 ± 1.6</td>
<td>28.0 ± 5.0</td>
<td>60.5 ± 5.9</td>
<td>81.2 ± 5.8</td>
</tr>
<tr>
<td>L-NAME+indomethacin</td>
<td>6.5 ± 2.2</td>
<td>32.3 ± 4.2</td>
<td>66.1 ± 4.8</td>
<td>85.0 ± 6.2</td>
</tr>
<tr>
<td>Mevalonate</td>
<td>5.7 ± 2.1</td>
<td>30.0 ± 9.7</td>
<td>57.6 ± 8.6</td>
<td>83.5 ± 8.2</td>
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Data are expressed as mean percentage of maximum dilation ± SEM in four to six experiments. Based on two-way ANOVA, responses to sodium nitroprusside were not affected by any perturbations compared with the control.

Figure 2. The role of endothelium in the retinal arteriolar dilation response to simvastatin. Dose-dependent vasodilation in response to simvastatin was examined before (control) and after removal of the endothelium by perfusing it with 0.4% CHAPS. *P < 0.05 versus control.

Figure 3. The role of endothelium-derived factors in the dilation of retinal arterioles in response to simvastatin. The dose-dependent vasodilation response to simvastatin was examined before (control) and after incubation with the NO synthase inhibitor L-NAME (10 μM), the cyclooxygenase inhibitor indomethacin (10 μM), or the cytochrome P450 inhibitor sulfaphenazole (10 μM). Residual vasodilation in the presence of L-NAME (10 μM) was examined after co-incubation with indomethacin (10 μM). n, number of vessels. *P < 0.05 versus control.
threshold concentration at 0.1 M elicits dose-dependent dilation of small retinal arterioles with a response that is consistent with that reported in rat aorta and mesenteric arteries. Therefore, it remains obscure whether statins exhibit a direct vasodilatory response to simvastatin was examined before (control) and after incubation with the soluble guanylyl cyclase inhibitor ODQ (0.1 μM). The role of guanylyl cyclase in the isolated retinal arteriolar dilation response to simvastatin was demonstrated for the first time that on the mesenteric artery and aorta (i.e., 25%–30% reduction in resistance arterioles). In contrast, the direct activation of smooth muscle by simvastatin is more evident at higher concentrations. The signaling pathway responsible for the endothelium-dependent vasodilation response to simvastatin in retinal arterioles is currently unclear. However, it has been shown that simvastatin could inhibit calcium release from intracellular pools and reduce extracellular calcium entry in denuded aortic rings. These findings suggest that simvastatin may reduce cytosolic calcium in smooth muscle cells and subsequently lead to vasodilation.

In the present study, we examined the possible role of endothelium-derived vasodilators such as NO, prostaglandins, and EDHF in simvastatin-induced vasodilation. We found that blockade of NOS greatly inhibited the simvastatin-induced response, suggesting that NO contributes in large part to the vasodilation (Fig. 3). The involvement of NO in simvastatin-induced vasodilation was also reported in isolated bovine coronary arteries and in rat aorta and mesentery arteries. Moreover, an increase in retinal blood flow associated with the elevated level of NO metabolites (i.e., nitrite/nitrate) in the human plasma after systemic administration of simvastatin has also been reported. Although this clinical study speculated that the released NO may contribute to the elevation of retinal blood flow, the source of nitrite/nitrate, and the actual role of NO in relation to retinal vascular activity were not determined.

The time course of the vasomotor response indicates that simvastatin elicits retinal arteriolar dilation within a few minutes (Fig. 1A). Although the beneficial effects of statins by increasing endothelial NOS (eNOS) expression and activity may take hours and days, recent reports have indicate that NO release from cultured endothelial cells can be elevated rapidly by statins in a matter of seconds or minutes. Of note, blockade of eNOS protein phosphorylation has been shown to attenuate acute statin-stimulated NO release from cultured endothelial cells, suggesting that the rapid increase in eNOS activity by phosphorylation may contribute to the acute release of NO. In the study of skeletal muscle microcirculation, intra-arterial infusion of simvastatin (10 mM) caused a marked dilation of small arterioles in 1 to 2 minutes. In the isolated heart preparation, intracoronary infusion of simvastatin (3–30 μM) produced an immediate increase in coronary blood flow, suggesting the rapid dilation of resistance arterioles. These responses were attenuated by an NOS inhibitor, indicating the involvement of NO in the vasomotor response to simvastatin.

Our data showed that endothelial disruption abolished vasodilation to simvastatin except at the highest concentration, suggesting a cardinal role for the endothelium in this vascular response. The observed partial endothelium-dependent response is consistent with that reported in rat aorta and mesenteric arteries, although there is a difference in the animal species, organ, and vessel size. It is worth noting that the removal of the endothelium had a greater impact on retinal arteriolar dilation (i.e., 65% reduction in dilation; Fig. 2) than that on the mesenteric artery and aorta (i.e., 25%–30% reduction in dilation). It seems that simvastatin preferentially acts to exert vasodilation on the endothelial cells in the small resistance arterioles. In contrast, the direct activation of smooth muscle by simvastatin is more evident at higher concentrations. The signaling pathway responsible for the endothelium-independent vasodilation response to simvastatin in retinal arterioles is currently unclear. However, it has been shown that simvastatin could inhibit calcium release from intracellular pools and reduce extracellular calcium entry in denuded aortic rings. These findings suggest that simvastatin may reduce cytosolic calcium in smooth muscle cells and subsequently lead to vasodilation.

The role of mevalonate and the Rho kinase pathway in the isolated retinal arteriolar dilation to simvastatin. The dose-dependent vasodilation in response to simvastatin was examined before (control) and after the intraluminal incubation with mevalonate (1 mM) and combination of mevalonate (1 mM) and the Rho kinase inhibitor Y-27632 (0.1 μM). *P < 0.05 versus control.
Simvastatin. Although the actual concentration of simvastatin in the vasculature is not known in those infusion studies, the results indicate that microvascular beds, including retinal arterioles as shown in the present study, can respond to simvastatin acutely by releasing NO.

Recent in vitro studies have demonstrated that the vessel relaxation response to simvastatin is attenuated by cyclooxygenase inhibitor in bovine coronary arteries and in rat aorta and mesenteric arteries. These studies, including ours, point to the participation of prostaglandins in the vasodilation reaction to simvastatin. However, the mechanism responsible for the cyclooxygenase-mediated vasodilation response to simvastatin is still unclear. The transient increase in endothelial calcium by simvastatin and the subsequent production of arachidonic acid by calcium-activated phospholipase A2 may lead to the synthesis of vasodilator prostacyclin through prostacyclin synthase. Recent studies have shown that prostacyclin synthase is susceptible to tyrosine nitration and that the latter process can be prevented by simvastatin in various tissues. Although all these findings are in different cells, tissues, and species under different biological environments and they may work differently from our experimental model, the action of simvastatin is seemingly in favor of prostacyclin synthesis. Indeed, the production of prostacyclin by endothelial cells can be enhanced by a low concentration of fluvastatin (i.e., 0.1 μM). It is possible that the acute release of prostacyclin contributed to the observed dilation in retinal arterioles. A recent in vivo assay suggested that the immediate action of simvastatin in antithrombin formation is attributable to the endothelial release of prostacyclin. It appears that the rapid release of endothelial factors—that is, NO and prostacyclin—not only contributes to the acute dilation of the microvessel but also protects the vascular wall from thrombigenic insults. Activation of the soluble guanylyl cyclase/cGMP pathway is generally considered to be a major vasodilatory mechanism for NO. However, recent studies have demonstrated that NO also elicits vasodilation through cGMP-independent pathways by activating potassium channels or gap junctions, enhancing calcium uptake by the sarcoplasmic reticulum, inhibiting 20-HETE formation and some yet unidentified mechanisms. Therefore, the signaling molecules responsible for the simvastatin-induced NO-mediated dilation in retinal arterioles remain to be determined. We have demonstrated for the first time that the selective soluble guanylyl cyclase/cGMP pathway is generally considered to be a major vasodilatory mechanism for NO. However, recent studies have demonstrated that NO also elicits vasodilation through cGMP-independent pathways by activating potassium channels or gap junctions, enhancing calcium uptake by the sarcoplasmic reticulum, inhibiting 20-HETE formation and some yet unidentified mechanisms. Therefore, the signaling molecules responsible for the simvastatin-induced NO-mediated dilation in retinal arterioles remain to be determined. We have demonstrated for the first time that the selective soluble guanylyl cyclase inhibitor ODQ reduced the dilation of retinal arterioles to simvastatin. Because the effect of ODQ was comparable to that produced by i-NAMe and the combination of ODQ and i-NAMe had no further inhibitory effect (n = 4, data not shown), these results indicate the pivotal role of soluble guanylyl cyclase/cGMP in mediating the NO-dependent dilation response to simvastatin. In addition to the release of NO and prostaglandins, epoxygenes atiothiéne acids derived from cytochrome P450 epoxygenase in the endothelium have been demonstrated to be the EDHF in various vascular beds. To the best of our knowledge, there has been no study to date to examine the role of EDHF in statin-induced vasodilation in the retinal circulation. In the present study, we found that the cytochrome P450 enzyme inhibitor sulphanaphene did not alter the dose–response curve for simvastatin, suggesting that the simvastatin-induced dilation in retinal arterioles is independent of EDHF. It has recently been reported that NO- but not EDHF-mediated relaxation of the mesenteric artery in hypertensive rats is improved by fluvastatin. In addition, lovastatin selectively maintained endothelium-dependent NO-mediated relaxation but not EDHF-mediated relaxation in hypercholesterolemic rabbit carotid arteries. It appears that EDHF contributes little, if any, to the statin-induced vasomotor activity and vascular protection in either normal or disease states.

The statins are known to inhibit HMG-CoA reductase for mevalonate synthesis, leading to reduced levels of cholesterol and other molecules such as isoprenoids for the posttranslational modification of several cell signaling molecules including small GTP-binding protein Rho. The mRNA stability and protein expression have been shown to be negatively regulated by Rho GTPase in cultured endothelial cells. Moreover, Rho/Rho kinase activation negatively regulates eNOS activity and NO production and suppresses NO-mediated vessel relaxation in intact aortic rings. The speculation has been that the simvastatin-induced NO-mediated dilation occurs as a result of the inhibition of the mevalonate-Rho pathway in retinal arterioles. This contention is supported by the present finding that intraluminal administration of mevalonate inhibits the effect of simvastatin. In addition, the blockade of Rho kinase antagonized the inhibitory action of mevalonate and preserved the simvastatin-induced dilation (Fig. 5). Of note, extraluminal administration of mevalonate was ineffective (n = 4, data not shown). The reason for detecting only the intraluminal effect of mevalonate is unclear. It has been suggested that the cellular uptake and metabolism of exogenous mevalonate can be influenced by various factors, including, but not limited to, its permeability, metabolic (isopentenyl/farnesyl) enzyme activity and intracellular concentration of mevalonate. In the present study, the selective effect of mevalonate may be related to the insufficient incubation time for its transport from abluminal to luminal side of the vessel and/or differential (smooth muscle cell versus endothelium) uptake/metabolism of mevalonate. Further studies are clearly needed to fully understand this issue. Nevertheless, the ability of intraluminal mevalonate to prevent the vasodilatory action of simvastatin suggests that the blockage of mevalonate-Rho pathway in the endothelium is the target for simvastatin. However, it should be noted that the dilation response to simvastatin, especially at the higher concentrations, was only partially inhibited by mevalonate. This result suggests that other mechanisms, independent of the mevalonate-Rho pathway, are involved in the regulation of vasomotor activity by simvastatin. A recent study showed that NO production in endothelial cells can be acutely (~1–5 minutes) increased by statins through eNOS phosphorylation by P3-kinase and protein kinase A, independent of HMG-CoA/ mevalonate. This finding explains the observed partial effect of mevalonate in our study. Future investigations on the relation between eNOS phosphorylation and vasodilation elicited by statins in intact retinal arterioles are needed to support this view.

In summary, the present study demonstrated that simvastatin elicits a marked dilation of retinal arterioles independent of its cholesterol-lowering effect. This dilation is mainly dependent on the endothelium and mediated by the activation of the guanylyl cyclase/cGMP pathway through released NO. It appears that inhibition of mevalonate and its subsequent pathway for Rho kinase activation is responsible, in part, for the vasodilation response to simvastatin in retinal arterioles. Understanding the mechanisms involved in the statin-stimulated NO release and vasomotor activity, in addition to its cholesterol-lowering effect, may help explain the recent clinical reports on the improvement of retinal blood flow and the beneficial outcomes after administration of statins in patients with ocular disorders such as glaucoma, age-related macular degeneration, and diabetic retinopathy. However, further clinical study is needed to evaluate the relative contribution of flow improvement to the alleviation of ocular disease by statins.

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