Brimonidine evokes heterogeneous vasomotor response of retinal arterioles: diminished nitric oxide-mediated vasodilation when size goes small

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Brimonidine evokes heterogeneous vasomotor response of retinal arterioles: diminished nitric oxide-mediated vasodilation when size goes small. Am J Physiol Heart Circ Physiol 291: H231–H238, 2006. First published February 17, 2006; doi:10.1152/ajpheart.01281.2005.—Brimonidine, an α2-adrenergic receptor (AR) agonist, has been employed in the treatment of glaucoma due to its beneficial effects on intraocular pressure reduction and neuroprotection. In addition, some studies have implicated that brimonidine might influence ocular blood flow; however, its effect on the retinal microcirculation has not been documented. Herein, we examined the vasomotor action of brimonidine on different branching orders of retinal arterioles in vitro and determined the contribution of the α2-AR subtype and the role of endothelium-derived nitric oxide (NO) in this vasomotor response. First- and second-order retinal arterioles of pigs were isolated, cannulated, and pressurized for functional studies. Videomicroscopic techniques were employed to record diameter changes and determined the contribution of the α2-AR subtype and the role of endothelium-derived nitric oxide (NO) in this vasomotor response. RT-PCR was performed for detection of α-AR and endothelial NO synthase (eNOS) mRNA in retinal arterioles. All first-order arterioles (82 ± 2 μm ID) dilated dose dependently to brimonidine (0.1 nM to 10 μM) with 10% dilation at the highest concentration. Second-order arterioles (50 ± 1 μm ID) responded heterogeneously with either dilation or constriction. The incidence and magnitude of vasoinhibition were increased with increasing brimonidine concentration. Administration of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester abolished the brimonidine-induced vasodilation in first- and second-order arterioles. Regardless of vessel size, vasomotor responses (i.e., vasodilation and vasoconstriction) of retinal arterioles were sensitive to the α2-AR antagonist rauwolscine. Consistent with the functional data, α2A-AR and eNOS mRNAs were detected in retinal arterioles. Collectively, our data demonstrate that brimonidine at clinical doses evokes a consistent NO-dependent vasodilation in first-order retinal arterioles but a heterogeneous response in second-order arterioles. These vasomotor responses are mediated by the activation of α2-AR. It appears that brimonidine, depending on the concentration and vessel size, may alter local retinal blood flow.

retinal blood flow; retinal microcirculation

BRIMONIDINE (Alphagan), an α2-adrenergic receptor (AR) agonist, has been employed in the clinical setting of glaucoma because of its therapeutic potential of reducing intraocular pressure (IOP) by aqueous suppression and enhanced uveoscleral outflow (8, 21). In addition to the IOP-lowering effects, several recent investigations in animal models suggest that brimonidine may exert a neuroprotective effect on retinal ganglion cells by preventing cell death secondary to ischemic injury (14, 18, 30). A potential mechanism of neuroprotection implicated in the literature involves the α2-AR agonist effect on the retinal and optic nerve head vasculature (25, 31, 36, 38, 49). Topical brimonidine administration has reportedly produced acute (12 h) and sustained (up to 6 mo) increases in pulsatile ocular blood flow in patients with primary open angle glaucoma (49) and normal tension glaucoma (36). Moreover, studies (25, 38) employing the Heidelberg retinal flowmeter also demonstrated an acute increase (90 min to 2 wk) in retinal blood flow after topical administration of brimonidine. Because brimonidine also reduces IOP, the increased retinal or ocular blood flow may be a consequence of IOP reduction and/or its association with alteration of ocular rigidity during pulsatile ocular blood flow measurements. In contrast to the above findings, other investigations utilizing either color Doppler imaging (24, 29, 41) or the Heidelberg retinal flowmeter (11, 40) have disclosed no significant acute and/or chronic hemodynamic effects of brimonidine on the retinal, choroidal, and/or optic nerve head circulation in humans.

Although a precise explanation for the above apparent discrepancies is not currently available, factors involving differences in patient demography and drug administration (frequency and duration), experimental/instrumental variations, and uncertainties in the concentration and distribution of the drug in retinal tissue should be considered. Furthermore, a heterogeneous vascular response to α2-AR agonists may exist in the ocular circulation. Nonetheless, it remains unclear whether brimonidine exhibits any vasomotor action on retinal microvessels. Herein, using isolated vessel approaches, we investigated the direct effect of brimonidine on retinal microvascular diameter and determined the contribution of the α2-AR subtype and the endothelium-derived vasodilator nitric oxide (NO) to the observed vasomotor reaction. We also examined the gene expression of α1- and α2-ARs and endothelial NO synthase (eNOS) in retinal arterioles.

MATERIALS AND METHODS

Animal preparation. All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Scott and White Institutional Animal Care and Use Committee. Pigs, 27 male and 5 female (8–12 weeks of age) responded heterogeneously with either dilation or constriction. The incidence and magnitude of vasoinhibition were increased with increasing brimonidine concentration. Administration of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester abolished the brimonidine-induced vasodilation in first- and second-order arterioles. Regardless of vessel size, vasomotor responses (i.e., vasodilation and vasoconstriction) of retinal arterioles were sensitive to the α2-AR antagonist rauwolscine. Consistent with the functional data, α2A-AR and eNOS mRNAs were detected in retinal arterioles. Collectively, our data demonstrate that brimonidine at clinical doses evokes a consistent NO-dependent vasodilation in first-order retinal arterioles but a heterogeneous response in second-order arterioles. These vasomotor responses are mediated by the activation of α2-AR. It appears that brimonidine, depending on the concentration and vessel size, may alter local retinal blood flow.
is a nitroarginine methyl ester (t-NAME) inhibitor. To confirm the efficacy of t-NAME, vasodilation to the NO-mediated agonist bradykinin (22) was examined. At the end of each isolated vessel study, a complete dose-dependent vasodilation to sodium nitroprusside was examined to ensure that the smooth muscle vasodilatory function was intact. The vessels were excluded from data analysis if the responsiveness to sodium nitroprusside was compromised. All drugs were administered extraluminally, and each antagonist (i.e., rauwolscine and t-NAME) was incubated for at least 30 min.

**Chemicals.** Drugs were obtained from Sigma-Aldrich (St. Louis, MO). Bradykinin, sodium nitroprusside, and t-NAME were dissolved in PSS. Rauwolscine and brimonidine were dissolved in water and DMSO, respectively, as stock solutions (10 mM), and subsequent concentrations were diluted in PSS. The final concentration of DMSO in the vessel bath was 0.1%. Vehicle control studies indicated that the final concentration of solvent had no effect on arteriolar function.

**RNA isolation and reverse transcription polymerase chain reaction.** Because low AR message level was detected in our preliminary studies, first- (6 to 8 per sample) and second-order retinal arterioles (12 to 16 per sample) were dissected under 6°C PSS and pooled together from both eyes for each RT-PCR experiment. These vessels were homogenized in 1 ml TRIzol reagent (Invitrogen, Carlsbad, CA), and total RNA was isolated according to the manufacturer’s instructions. Because the α2A-AR subtype is the predominant α2AR in porcine and human retina (9, 50), we determined whether retinal arterioles express this AR subtype. Porcine neural retina tissue was used as a positive control for α2A-AR mRNA. Sets of primers specific for the α1AR (gene accession no. U03864, sense: 5′-CAA AGT CTC CAG CCT GTC GCA CAA-3′, antisense: 5′-ATC GGT CCT CCG TAG GTT GCT GTA-3′), α2A-AR (gene accession no. NM000681, sense: 5′-GGT TCC CCT TCT TCT TCA CTA-3′, antisense: 5′-TCG TGG TTG AAG ATG GTG TAG ATG-3′), eNOS (gene accession no. M93718, sense: 5′-GGT CCT GCC GGT CTC ACC ACC-3′, antisense: CTC CGG GAA AAA GCT CTT-3′), and GAPDH (gene accession no. U48832, sense: 5′-CCA CCC ACC GCA AGT TCC ACC ACC-3′, antisense: 5′-GGT GGT GCA GGA GGC ATG ATT GCT GAC-3′) genes were engineered (Sigma-Genosys, The Woodlands, TX), and RT-PCR was constructed. With the use of 0.1 and 1 μg/μl of total RNA for eNOS/GAPDH and α-AR samples, respectively, RT (Thermoscript reverse transcriptase, Invitrogen) and PCR (Expand High Fidelity PCR enzyme, Roche, Indianapolis, IN) reactions were conducted as delineated previously (51). To determine whether the PCR reaction was amplifying genomic DNA, a first-strand cDNA synthesis reaction was performed with and without RT. The PCR reaction was optimized and run for 40 cycles for α1-AR, α2A-AR, and eNOS genes and 35 cycles for GAPDH genes. The PCR-amplified products were electrophoresed on a 1.8% agarose gel and visualized with ethidium bromide staining. Images of ethidium bromide-stained products were acquired with the Gel Doc 1000 system (Bio-Rad, Hercules, CA) and quantified by using volume integration (Multi-Analyst software/Macintosh, Bio-Rad). The level of expression of α2A-AR transcripts was normalized to that of GAPDH transcripts.

**Data analysis.** At the end of each functional experiment, the vessel was relaxed with 0.1 mM sodium nitroprusside in ethylenediaminetetraacetic acid (1 mM)-calcium-free PSS to obtain its maximal diameter at 55 mmHg intraluminal pressure. For the analysis of brimonidine-induced responses, diameter changes were normalized to the resting diameter and expressed as a percent change in resting diameter. Data are reported as means ± SE, and the n value represents the number of vessels (one per pig) studied. Statistical comparisons of data were performed by Student’s t-test or by analysis of variance followed by the Bonferroni multiple-range test, as appropriate. Regression analysis and Pearson correlation were performed to assess the relationship between resting diameter and percent change in
resting diameter at various concentrations of brimonidine. A value of $P < 0.05$ was considered significant.

**RESULTS**

Vasomotor response to brimonidine. First-order ($n = 18$) and second-order ($n = 16$) retinal arterioles developed a comparable level of basal tone (first-order: constricted to $67 \pm 1\%$ of their maximal diameter vs. second-order: constricted to $63 \pm 2\%$ of their maximal diameter; $P = 0.09$) at a bath temperature of $36–37^\circ C$ and $55\text{ cmH}_2\text{O}$ intraluminal pressure.

The average resting diameters of the first- and second-order arterioles were $82 \pm 2\mu m$ and $50 \pm 1\mu m$, respectively. Figure 1 shows the relationship between resting diameter and individual vascular response to various concentrations of brimonidine. The slope and the correlation coefficient ($r$) for the diameter-response relationship were increased with increasing brimonidine concentrations, indicating a size-dependent vasomotor response. The plot of individual responses revealed that a majority of first-order arterioles ($66–96\mu m$) dilated to brimonidine at all concentrations. At downstream second-order

![Figure 1](http://ajpheart.physiology.org/)

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**Fig. 1.** Scatterplots showing relationship between resting diameter and percent change in resting diameter at various concentrations of brimonidine. Vasomotor responses are plotted for individual first-order ($n = 18$) and second-order ($n = 16$) retinal arterioles. Solid line, regression analysis; vertical dashed line, separation between first-order ($>60\mu m$ ID; closed circles) and second-order ($\leq 60\mu m$ ID; open circles) retinal arterioles; horizontal dashed line, separation between vasodilation (positive change in resting diameter) and vasoconstriction (negative change in resting diameter); $r$, correlation coefficient.
arterioles (42–60 μm), lower concentrations of brimonidine (≤10^{-8} M) diluted ~30% of the vessels. However, these vessels constricted consistently to higher concentrations of brimonidine (≥10^{-7} M). Brimonidine elicited a reproducible dose-dependent dilation and constriction of first-order (Fig. 2A) and second-order (Fig. 2B) arterioles, respectively. To determine the contribution of α2-AR in these vascular responses, some vessels were treated with the α2-AR antagonist rauwolscine and the dose-dependent responses to brimonidine were reexamined. Rauwolscine did not significantly alter basal tone of first-order (control: 65 ± 2% vs. rauwolscine: 67 ± 4%) or second-order (control: 66 ± 3% vs. rauwolscine: 63 ± 4%) arterioles but blocked the respective dilation (Fig. 2A) and constriction (Fig. 2B) of these vessels to brimonidine. In contrast to the varied response to brimonidine, dose-dependent vasodilation to the endothelium-independent agonist sodium nitroprusside was not significantly different between first- and second-order retinal arterioles (Fig. 3A). Furthermore, rauwolscine did not affect basal tone (first-order arterioles, control: 66 ± 3% vs. rauwolscine: 68 ± 2%; second-order arterioles, control: 60 ± 2% vs. rauwolscine: 60 ± 2%) or sodium nitroprusside-induced dilation (Fig. 3B), indicating that vasodilatory function of this group of vessels was not compromised by the α2-AR antagonist.

**Role of endothelium-derived NO in retinal arteriolar dilation to brimonidine.** The relative contribution of endothelium-derived NO to brimonidine-induced vasodilation of first-order retinal arterioles was examined in the absence and presence of L-NAME, a NO synthase inhibitor. L-NAME had a tendency to increase basal tone (control: 67 ± 2% vs. L-NAME: 64 ± 3%; P = 0.13) but not in a significant manner. The retinal arteriolar dilation to an endothelium-dependent, NO-mediated agonist bradykinin (10 nM) was significantly inhibited by L-NAME.
(control: 80 ± 5% of maximal dilation vs. l-NAME: 26 ± 5% of maximal dilation; n = 4). Similar to rauwolscine, l-NAME completely blocked the vasodilatory response to brimonidine in first-order arterioles (Fig. 4). The dilation of retinal arterioles to the endothelium-independent NO donor sodium nitroprusside was not altered by l-NAME (data not shown), which is consistent with our previous findings (22).

α1-AR and eNOS mRNA expression in retinal arterioles. To support the results from functional studies above, we performed RT-PCR to detect α1-AR and eNOS mRNAs in isolated retinal arterioles and neural retina tissue. Figure 5 shows that the α2A-AR and eNOS transcripts, but not the α1-AR transcript, were detected in retinal arterioles. In contrast, both α2A-AR and α1-AR transcripts were detected in the neural retina tissue devoid of retinal vessels. The expression of α2A-AR mRNA, after normalization with GAPDH, was consistently slightly higher in retinal arterioles than in neural retina tissue (Fig. 5). It appears that the retinal arteriole sample was relatively homogeneous because α1-AR was observed only in the neural retina tissue.

DISCUSSION

In the present study, with an established videomicroscopic technique (22), we examined the direct action of brimonidine on isolated porcine retinal arterioles. Brimonidine caused dose-dependent dilation of all first-order retinal arterioles and mild dilation of some second-order retinal arterioles at the low concentrations (≤10 nM) (Fig. 1). It should be noted that this vasodilatory response was evident at therapeutic concentrations (i.e., vitreous concentrations ∼10–100 nM after topical administration) (1, 26). At these presumed therapeutic doses, brimonidine produced up to an 8% increase in resting diameter of first-order retinal arterioles (Figs. 2A and 4) and up to a 10% decrease in diameter in second-order arterioles (Fig. 2B). This magnitude of vasodilation or vasoconstriction would have a significant impact on local retinal blood flow, because Poiseuille’s law indicates that blood flow, at a constant driving pressure, is directly proportional to the fourth power of the vessel radius.

To our knowledge, only one other study has evaluated the retinal vasomotor reactivity associated with brimonidine. Spada et al. (42) studied the effect of locally administered brimonidine on microvessel caliber with intravital microscopy in human retinal tissues grafted into the hamster cheek pouch. These investigators found that brimonidine (1 nM to 0.1 mM) caused dose-dependent constriction in the microvessels of the naive hamster cheek pouch but did not cause significant constriction or dilation of selected arteriolar segments (∼12–25 μm in resting diameter) in human retinal xenografts. Only a transient (1 min) and modest vasoconstriction (∼10%) was observed when a high concentration of brimonidine (0.1 mM) was administered. Although Spada et al. (42) did not investigate vasomotor response in larger arterioles, their results support our findings on the increased incidence of vasoconstriction to a high concentration of brimonidine in smaller retinal arterioles. It is worth mentioning that the growth of new vessels in the xenograft, confounding influences from hemodynamic factors, and the potential release of local vasomotor chemicals from hamster native tissue might play a role in modulating brimonidine-induced vasoreactivity in the above hamster cheek pouch preparation. In our study, we obviated these potential confounding influences by characterizing the vasomotor reactivity to brimonidine in a controlled environment using the isolated vessel preparation.

Interestingly, Hughes et al. (23) found size-dependent response of isolated resistance arteries to brimonidine in human splanchnic, peripheral, coronary, pulmonary, and uterine tissue. The magnitude of the contractile response was reported to be inversely related to vessel size (23). Our findings suggest that the retinal arteriolar network exhibits a unique size-
dependent vasomotor response to brimonidine with homogeneous vasodilation in first-order arterioles and a heterogeneous vasomotor reaction in second-order arterioles with predominantly vasoconstriction at higher concentrations. In this regard, the effect of brimonidine on overall retinal blood flow seemingly depends on the local concentration and the reaction of large versus small arterioles to this drug. It appears that a general increase in retinal blood flow is expected at lower doses of brimonidine due to dilation of first- and second-order arterioles. However, this increased flow might be counterbalanced or even reversed by the increased vasoconstriction in second-order arterioles when local concentration of brimonidine is further elevated. This may explain the apparent inconsistent results of retinal arterial blood flow in response to brimonidine in vivo because the size of vessels studied was inconsistent and the concentration and distribution of brimonidine in retinal tissue were uncertain during flow/velocity measurement. Moreover, because retinal tissue exhibits autoregulation of blood flow (16), the initiation of compensatory vasoregulatory mechanisms secondary to flow alteration by brimonidine cannot be excluded. Nevertheless, our present study demonstrates that brimonidine, at a clinical concentration, is vasoactive in retinal microvessels.

Despite the apparent lack of autonomic innervation in the human retinal vasculature, $\alpha_1$- and $\alpha_2$-ARs (9, 33, 50) have been shown to localize to the retina, and topically applied brimonidine can reach the posterior segment of the eye at nanomolar concentrations sufficient to activate $\alpha_2$-AR (1, 26). However, the expression of $\alpha_2$-ARs in retinal arterioles has not been reported, and the recent observation on the insignificant retinal flow response to brimonidine has raised the question of whether retinal arterioles express $\alpha_2$-AR (41). In the present study, we demonstrated for the first time that $\alpha_2\Delta_2$-AR mRNA is expressed in retinal arterioles (Fig. 5). This is consistent with the report that $\alpha_2\Delta_2$-AR subtype is the predominant subtype in the porcine and human retinal tissue (9, 50), supporting our use of the porcine model to investigate the effect of brimonidine on the retinal vasculature.

Activation of $\alpha_2$-AR can cause constriction of arterioles in the skeletal muscle microcirculation in human beings (39) and in some animal models (10, 35). However, vasodilation in response to $\alpha_2$-AR activation in mesenteric (10), coronary (6), and cerebral (47) vessels was also reported. Because endothelial removal or inhibition of NO synthase can augment vasoconstriction induced by $\alpha_2$-AR agonists, it has been suggested that endothelial release of NO (via endothelial $\alpha_2$-AR activation) can modulate the contractile action of $\alpha_2$-AR in smooth muscle cells (2, 48). In fact, it has been shown in a number of vascular beds that activation of $\alpha_2$-AR by brimonidine is associated with NO-mediated vasodilation (5, 43, 47). Moreover, endothelial release of vasodilator prostaglandins or hyperpolarizing factors also has been reported in some vasculatures (46). However, the vasomotor signaling pathways involved in $\alpha_2$-AR activation by brimonidine in retinal arterioles have not been determined. In the present study, we found that rauwolscine, an $\alpha_2$-AR antagonist, abolished brimonidine-induced vasodilation in both first- (Fig. 2A) and second-order ($n = 3$; data not shown) retinal arterioles and also blocked vasoconstriction in small arterioles (Fig. 2B). These results indicate that $\alpha_2$-AR activation is responsible for the vasomotor action of brimonidine in retinal arterioles. In addition, the NO synthase inhibitor L-NAME completely blocked the vasodilatory response to brimonidine in both first- (Fig. 4) and second-order ($n = 3$; data not shown) retinal arterioles, suggesting that $\alpha_2$-AR activation stimulates NO release and is mechanistically involved in the dilation of retinal arterioles to brimonidine. It does not appear that this NO release is sufficient to compete with the brimonidine-elicted constrictor response because L-NAME did not enhance constriction of second-order arterioles to brimonidine ($n = 3$; data not shown). In contrast to in vivo findings in the retinal circulation (15), it is important to note that L-NAME (10 $\mu$M) did not significantly increase basal tone of isolated retinal arterioles. This may be due to the absence of luminal flow in our in vitro study because it has been shown that endothelial cells respond to increased flow (or shear stress) by releasing NO (28). In this regard, it is expected that the NO component would be more pronounced in vivo (i.e., with luminal flow) compared with that in vitro (i.e., without luminal flow) under resting conditions. Therefore, the effect of L-NAME on basal vascular tone would be less apparent as observed in our present and previous (22) in vitro studies. The possible role of endothelial NO signaling in response to agonist stimulation was supported by our molecular evidence that eNOS mRNA was detected in the retinal arterioles. Because brimonidine did not elicit or enhance vasoconstriction of retinal arterioles in the presence of rauwolscine, it is unlikely that brimonidine has other vasomotor activity through activation of $\alpha_1$-AR besides $\alpha_2$-AR. This contention is supported by the inability of the $\alpha_1$-AR agonist phenylephrine to alter resting diameter of first- or second-order retinal arterioles ($n = 3$; data not shown), as well as the identification of $\alpha_2$-AR, but not $\alpha_1$-AR, mRNA expression in isolated retinal arterioles.

The vasocostrictor action of brimonidine in second-order retinal arterioles is expected to compromise local retinal blood flow. However, it is worth noting that arteriolar constriction to $\alpha_2$-AR activation is selectively inhibited by moderate tissue acidosis (12), flow reduction (34), hypoxia (44), increased tissue metabolic rate (3), and by release of adenosine (13). The prevailing inhibition of adrenergic $\alpha_2$-mediated constriction in second-order arterioles under metabolic stress might help to alleviate the impact of brimonidine-induced constriction in small retinal arterioles and consequently facilitate flow distribution to the stressed tissues. On the other hand, because accumulating evidence suggests that endothelin-1 can either prevent $\alpha_2$-AR-mediated NO release and vasodilation (4, 32) or reduce smooth muscle sensitivity to NO (19), the enhanced endothelin-1 production during disease states, such as glaucoma (17, 37, 45), may compromise the vasodilatory effect of brimonidine. It is reasonable to speculate that the beneficial effect of brimonidine, in terms of vasodilation and flow enhancement, may be dependent on the local concentration of brimonidine, distribution and function of $\alpha_2$-AR, endothelial NO synthase activity, endothelin-1 level, and the local metabolic environment.

In conclusion, we found molecular and pharmacological evidence of $\alpha_2$-ARs in the retinal arterioles. The $\alpha_2$-AR agonist brimonidine, at clinically relevant doses, evoked a modest vasodilatory response in first-order, and some second-order, porcine retinal arterioles via activation of $\alpha_2$-ARs and subsequent production of the vasodilator NO. Vasoconstriction of most second-order arterioles in response to brimonidine was also observed. This apparent heterogeneity of vascular re-
sponses implicates a potential influence of topical brimonidine administration on local retinal blood flow.

REFERENCES


