Identification of the Muscarinic Acetylcholine Receptor Subtype Mediating Cholinergic Vasodilation in Murine Retinal Arterioles

Adrian Gericke,1 Jan J. Sniatecki,1 Evgeny Goloborodko,1 Andreas Steege,2 Olga Zavaritskaya,3 Jan M. Vetter,1 Franz H. Grus,1 Andreas Patzak,4 Jürgen Wess,5 and Norbert Pfeiffer1

PURPOSE. To identify the muscarinic acetylcholine receptor subtype that mediates cholinergic vasodilation in murine retinal arterioles.

METHODS. Muscarinic receptor gene expression was determined in murine retinal arterioles using real-time PCR. To assess the functional relevance of muscarinic receptors for mediating vascular responses, retinal vascular preparations from muscarinic receptor–deficient mice were studied in vitro. Changes in luminal arteriole diameter in response to muscarinic and nonmuscarinic vasoactive substances were measured by video microscopy.

RESULTS. Only mRNA for the M3 receptor was detected in retinal arterioles. Thus, M3 receptor–deficient mice (M3R−/−) and respective wild-type controls were used for functional studies. Acetylcholine concentration–dependently dilated retinal arterioles from wild-type mice. In contrast, vasodilation to acetylcholine was almost completely abolished in retinal arterioles from M3R−/− mice, whereas responses to the nitric oxide (NO) donor nitroprusside were retained. Carbachol, an acetylcholineesterase-resistant agonist of acetylcholine, also evoked dilation in retinal arterioles from wild-type, but not from M3R−/−, mice. Vasodilation responses from wild-type mice to acetylcholine were negligible after incubation with the non–subtype-selective muscarinic receptor blocker atropine or the NO synthase inhibitor Nω-nitro-L-arginine methyl ester, and were even reversed to contraction after endothelial damage with 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate.

CONCLUSIONS. These findings provide evidence that endothelial M3 receptors mediate cholinergic vasodilation in murine retinal arterioles via activation of NO synthase. (Invest Ophthalmol Vis Sci. 2011;52:7479–7484) DOI:10.1167/iovs.11-7370

Muscarinic acetylcholine receptors are involved in various physiologic actions in the eye, such as regulation of intraocular pressure, pupil size, and ocular growth. Moreover, muscarinic receptors were shown to mediate acetylcholine–induced vasodilation in retinal blood vessels.2,3 The contribution of individual muscarinic receptor subtypes to vasodilation responses in retinal arterioles, however, remains unknown at present.

Five muscarinic receptor subtypes, M1–M5, have been identified.4 These receptors are generally grouped according to their preferential signaling pathways, either to the mobilization of intracellular calcium via activation of phospholipase Cβ (M1, M3, M5) or to the inhibition of adenyl cyclase (M2, M4).5 In most vascular beds, activation of muscarinic receptors induces powerful vasodilation via the release of vasorelaxing agents from the endothelium.6–8 Remarkably, the expression pattern of muscarinic receptor subtypes and their role in mediating vascular responses differ substantially between individual vascular beds.9–11 Previous studies aimed at identifying the muscarinic receptor subtypes involved in regulation of ocular perfusion suggested that the M3 receptor mediates cholinergic vasodilation in the choroid of pigeons and in ophthalmic arteries of mice.15,16 Thus, from a clinical point of view, the M3 receptor may represent a pharmacologic target to modulate ocular vascular tone. However, before the potential clinical usefulness of this approach can be pursued further, it is important to identify the muscarinic receptor subtype mediating cholinergic responses in retinal arterioles. Thus, we used real-time PCR to determine mRNA expression of all five muscarinic receptor subtypes in murine retinal arterioles. Given the lack of highly selective agonists and antagonists for individual muscarinic receptor subtypes, we used muscarinic receptor knockout mice to perform functional studies in vascular preparations from the retina.

MATERIALS AND METHODS

Animals

All experiments were performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local government. Experiments were performed in M3 receptor knockout mice (M3R−/−) and in respective wild-type controls. The generation of M3R−/− mice has been described previously.15 Briefly, the M3 receptor gene was inactivated using mouse embryonic stem cells derived from 129SvEv mice. The resulting chi-
meric mice were then mated with CF-1 mice to generate M3R−/− and wild-type mice with the following genetic contribution: 129SvEv (50%) × CF-1 (50%). The genotype of each mouse was determined by PCR of DNA isolated from tail biopsies. In all experiments, male mice ranging in age from 4 to 6 months were used.

**Real-Time PCR Analysis**

Muscarinic receptor gene expression was determined in isolated retinal arterioles from wild-type mice using real-time PCR. Since all five muscarinic receptor subtypes are known to be expressed in the mouse brain, we used RNA from the whole brain of wild-type mice as positive control. After mice had been killed by CO2 inhalation, a midline abdominal and thoracic incision was made. Then, the inferior vena cava was cut and the left cardiac ventricle cannulated with a needle that was connected to a silicon tube, and perfused with 10 mL of phosphate-buffered solution (PBS; Invitrogen, Karlsruhe, Germany) followed by an injection of iron oxide suspension (1% in 20 mL of PBS). The injected iron oxide microparticles are trapped in retinal arterioles, thereby enabling their visualization and differentiation from venules. Former studies in rabbit renal microvessels revealed no evidence of morphologic vascular damage by injection of iron oxide particles. After this procedure, the eyes were immediately removed and placed in ice-cold PBS. Subsequently, the retina was removed from the eye under a dissecting microscope and retinal arterioles were carefully isolated by using fine-point tweezers, transferred into a 1.5-mL tube, and immediately snap frozen. To increase RNA yield, arterioles from five different mice were pooled. Subsequently, vessels were homogenized in lysis buffer using a homogenizing device (FastPrep; MP Biomedicals, Illkirch, France). After homogenization, total RNA was extracted with a commercial kit (Absolutely RNA Nanoprep; Stratagene, La Jolla, CA) according to the manufacturer’s protocol.

After complete DNA digestion, the RNA was reverse transcribed with the use of a reverse-transcription kit (Superscript; Invitrogen, Karlsruhe, Germany) and random hexamers. Quantitative PCR analysis was performed (GeneAmp StepOne Plus; Applied Biosystems, Darmstadt, Germany). Nucleic acid stain (SYBR Green; Bioline, Luckenwalde, Germany) was used for the fluorescent detection of DNA generated during PCR. The PCR reaction was performed in a total volume of 12.5 μL with 0.4 pmol/μL of each primer and 2X ready-to-use reaction mix (ImmoMix; Bioline); 2 μL cDNA corresponding to 10 ng RNA was used as template. Published sequences for mouse M1 were used to design primers for PCR amplification. Primer sequences were M1 sense 5'-GAA GCC AGT CAA GAA TGC-3' and antisense 5'-AAT CAT CAG GCA TGC CCT GCG G-3'; M2 sense 5'-GGG ACC ACA AAA ATG GCA TTC-3' and antisense 5'-CCA TCA CCA CCA GGC ATG TTG TTG T-3'; M3 sense 5'-CCT CTT GAA GTG CTG CTG CTT GAC CTA G-3' and antisense 5'-TGC CAG GAA GGC AGT CAA GAA TGC-3'; M4 sense 5'-CTT TGG GTC GTC GTC GTC GTC-3' and antisense 5'-TGC CAG GAA GGC AGT CAA GAA TGC-3'; M5 sense 5'-CTT TGG GTC GTC GTC GTC GTC-3' and antisense 5'-TGC CAG GAA GGC AGT CAA GAA TGC-3'.

**Measurements of Vascular Reactivity**

Due to the small size of murine retinal arterioles (inner diameter < 30 μm), we established a method that allowed us to measure vascular reactivity in the mouse retina without the need of isolation and cannulation of individual vessels. A similar preparation has been previously used by Kulikarni et al. to measure vascular reactivity in the bovine retina. After mice had been killed by CO2 inhalation, the eyes were immediately removed, together with the retrobulbar tissue, and were placed in ice-cold Krebs buffer with the following ionic composition (in mM): 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, and 11 glucose (Carl Roth GmbH, Karlsruhe, Germany). Then, the retrobulbar tissue was removed under a dissecting microscope using fine-point tweezers and microsurgical scissors, taking care that the ophthalmic artery was not damaged. Once the surrounding tissue was removed, the ophthalmic artery and the orbital branches were ligated at the open ends with a 100 nylon monofilament suture. To exclude the possibility that responses of the ophthalmic artery to vasoactive substances applied during the experiment might affect retinal arteriolar diameter, we deactivated the retrobulbar vasculature by immersing the intact eye globe for 10 seconds into 70% ethanol followed by an intensive wash in cold Krebs buffer. In previous tests, we observed that proximal and distal segments of the ophthalmic artery lost the ability to contract in response to KCl (10−3 M) and the thromboxane mimetic 9,11-di-deoxy-9a,11b-methanoepoxy prostaglandin F2α (U-46619, 10−6 M) following this procedure. Moreover, intracellular Ca2+ transients in response to acetylcholine (10−5 M) and U-46619 (10−6 M) were absent in ethanol-treated segments of the ophthalmic artery loaded with the Ca2+-sensitive dye fura-2. Thus, it is very unlikely that the ophthalmic artery exerted a significant effect on the retinal vasculature during the experiments.

Subsequently, the globe was opened and the cornea, iris, scera, and lens were removed. A piece of sclera around the optic nerve was left to prevent injury and torsion of the very delicate distal part of the ophthalmic artery. Then, the preparation was placed into an organ chamber filled with cold Krebs buffer and the ophthalmic artery was cannulated with a glass micropipette and secured with 104 nylon monofilament suture. After this procedure, the retina was placed onto a transparent plastic platform, which had an indentation to fit the optic nerve and the retrobulbar blood vessels (Fig. 1), and four radial incisions were made, which enabled us to spread out the retina. Then, a stainless steel ring was placed onto the retina to fix it to the bottom (Fig. 1). Afterward, retinal vessels were pressurized via the micropipette located in the ophthalmic artery using a reservoir filled with Krebs solution to a level corresponding to 50 mm Hg and imaged.
under brightfield conditions using a video camera (TK-C1381; JVC Deutschland GmbH, Friedberg, Germany) mounted on an upright microscope (Olympus Vanox-T AH-2; Olympus Deutschland GmbH, Hamburg, Germany). The magnification resulted from a $100$ water-immersion objective lens (LUMPlanFL, 1.0 numerical aperture; Olympus Deutschland GmbH) and a 2.5 projection on a 0.5-inch chip digital camera (TK-C1381; JVC Deutschland GmbH). The spatial resolution of the system was 11 pixels/μm. Video sequences were captured to a personal computer for analysis. The organ chamber was continuously circulated with oxygenated and carbonated Krebs buffer at 37°C and pH 7.4. For our studies, we used retinal arterioles of the first order. Differentiation between arterioles and venules was made by morphology (arterioles were of smaller diameter than that of venules) and by the flow direction of the remaining red blood cells (centripetal in arterioles and centripetal in venules) directly after pressurizing the vessels.

After 30 minutes of equilibration, arterioles were preconstricted with the thromboxane mimetic U-46619 (5 × 10⁻⁶ to 10⁻⁵ M; Cayman Chemical, Ann Arbor, MI) to 50–70% of the initial vessel diameter. Then, responses of retinal arterioles from wild-type and M3R⁻/⁻ mice to acetylcholine (10⁻⁶ to 10⁻⁵ M; Sigma-Aldrich, Munich, Germany) and to the nitric oxide (NO) donor sodium nitroprusside (10⁻⁸ to 10⁻⁶ M; Sigma-Aldrich) were tested by cumulative application of the respective substances into the organ chamber. To exclude the possibility that potential differences in retinal acetylcholinesterase activity between wild-type and M3R⁻/⁻ mice affected acetylcholine-induced vascular reactivity, we additionally examined responses to carbachol (10⁻⁵ M; Sigma-Aldrich), an acetylcholinesterase-resistant analog of acetylcholine. To further examine the underlying mechanisms of cholinergic vasodilation of retinal arterioles, we conducted analogous studies in isolated retinal arterioles from five wild-type mice of the same genetic background as that used in the previous study. Since all five muscarinic receptor subtypes are known to be expressed in mouse brain, we used mRNA from the whole brain as a positive control. As expected, all five muscarinic receptor subtypes were detected in the brain. However, mRNA of the M3 receptor subtype only was found to be expressed in retinal arterioles. The values are averages of triplicate measurements and expressed as mean ± SE.

**RESULTS**

**Muscarinic Receptor mRNA Expression in Retinal Arterioles**

By the use of real-time PCR, we recently demonstrated that murine ophthalmic arteries expressed mRNA of all five muscarinic receptor subtypes.¹⁰ Using the same primers, we conducted analogous studies in isolated retinal arterioles from five wild-type mice of the same genetic background as that used in the previous study. Since all five muscarinic receptor subtypes were known to be expressed in mouse brain, we used mRNA from the whole brain as a positive control. As expected, all five muscarinic receptor subtypes were detected in the brain. Remarkably, we detected mRNA for the M3 receptor subtype in retinal arterioles (Fig. 2).

**Vascular Responses**

To examine whether M1 receptors play a role in mediating cholinergic vasodilation of retinal arterioles, we conducted functional studies in retinal vascular preparations from M3R⁻/⁻ and wild-type mice. Baseline luminal diameters of first-order retinal arterioles (before preconstriction) were 16.2 ± 1.3 μm (n = 6) and 17.1 ± 1.4 μm (n = 6) in M3R⁻/⁻ and wild-type mice, and did not differ between the two genotypes. Acetylcholine (10⁻⁸ to 10⁻⁵ M) elicited dose-dependent dilation in retinal arterioles from wild-type mice. The maximal increase in luminal artery diameter to 10⁻⁵ M acetylcholine was 26 ± 4% (n = 6) (Fig. 3A). In contrast, retinal arterioles from M3R⁻/⁻ mice showed almost no reactivity to acetylcholine. A negligible dilation of 1 ± 2% (n = 6) was observed at a concentration of 10⁻⁵ M (**P < 0.01, M3R⁻/⁻ vs. wild-type mice; Fig. 3A). To examine whether deletion of the M1 receptor gene affected responsiveness of vascular smooth muscle to NO, we examined responses of retinal arterioles to the NO

**Statistical Analysis**

Data are presented as means ± SE. Vascular responses are presented as percentage of change in luminal diameter, and n represents the number of mice per group. Statistical significance among concentration–response curves was calculated using the Brunner test for nonparametric analysis of longitudinal data. The Mann–Whitney test was applied for comparison of single-dose responses of retinal arterioles from wild-type and M3R⁻/⁻ mice to carbachol and to compare responses of CHAPS-treated with vehicle-treated retinal arterioles to acetylcholine and nitroprusside. For comparison of responses to acetylcholine before and after incubation with atropine or L-NAME, the Wilcoxon signed-rank test was used. A value of P < 0.05 was defined as significant.
donor nitroprusside. Nitroprusside (10^{-8} to 10^{-5} M) produced concentration-dependent dilation of retinal arterioles that did not differ between wild-type and M3R^{-/-} mice (Fig. 3B). Maximal vasodilation to 10^{-5} M nitroprusside was 47 ± 6% (n = 6) and 42 ± 6% (n = 6) in wild-type and M3R^{-/-} mice, respectively.

To exclude the possibility that differences in retinal acetylcholinesterase activity between wild-type and M3R^{-/-} mice affected vascular responses to acetylcholine, we examined responses of retinal arterioles to carbachol, an acetylcholine analog, which is resistant to degradation by acetylcholinesterase. Similar to acetylcholine, carbachol (10^{-5} M) induced pronounced vasodilation in arterioles from wild-type mice (23 ± 5%, n = 6), but only an insignificant increase in retinal arteriole diameter in M3R^{-/-} mice (3 ± 3%, n = 6; *P < 0.01, M3R^{-/-} vs. wild-type mice; Fig. 4).

To confirm that cholinergic responses in the retinal circulation were mediated by muscarinic receptors, we tested responses of retinal arterioles from wild-type mice to acetylcholine before and after addition of atropine (10^{-5} M), a non-subtype-selective muscarinic receptor blocker. After atropine treatment, responses to acetylcholine were negligible (29 ± 6% vs. 4 ± 3%, n = 5; *P < 0.05, nontreated versus treated; Fig. 5A). To identify the postreceptor mechanisms by which acetylcholine mediates vasodilation in the retina, we tested responses of retinal arterioles from wild-type mice to acetylcholine before and after incubation with the NO synthase inhibitor L-NAME (10^{-5} M). Remarkably, L-NAME almost completely abolished acetylcholine-induced vasodilation in retinal arterioles. Changes in luminal diameter were 27 ± 5% and −2 ± 6% in nontreated and treated arterioles, respectively (n = 5; *P < 0.05, nontreated versus treated; Fig. 5B). To test whether cholinergic vasodilation was endothelium dependent in the retina, we tested responses to acetylcholine and to the NO donor nitroprusside in retinal arterioles from wild-type mice that had been perfused with CHAPS, to damage the endothelium, and compared them with responses of vehicle-treated arterioles. Remarkably, retinal arterioles that had been treated with CHAPS did not dilate but contracted in response to acetylcholine. Changes in luminal diameter were 28 ± 8% and −13 ± 6% in vehicle-treated and CHAPS-treated arterioles, respectively (n = 5 per group; **P < 0.01, vehicle-treated versus CHAPS-treated; Fig. 6A). In contrast, nitroprusside elicited pronounced vasodilation that was similar in vehicle-treated and CHAPS-treated arterioles (36 ± 7% vs. 31 ± 5%, n = 5; *P > 0.05, vehicle-treated versus CHAPS-treated; Fig. 6B).

**FIGURE 4.** Responses of retinal arterioles from wild-type and M3R^{-/-} mice to carbachol. Vasodilation in response to carbachol (10^{-5} M) was almost completely abolished in retinal arterioles from M3R^{-/-} mice. Values are expressed as mean ± SE (n = 6 per genotype; **P < 0.01, M3R^{-/-} vs. wild-type mice).

**FIGURE 5.** Responses of retinal arterioles from wild-type mice to acetylcholine (10^{-5} M) before and after incubation with the non-subtype-selective muscarinic receptor blocker atropine (10^{-5} M) (A) and the NO synthase inhibitor L-NAME (10^{-5} M) (B). Remarkably, vasodilation responses were negligible after incubation with either of the two blockers. Values are expressed as mean ± SE (n = 5 per group; *P < 0.05, treated versus nontreated).

**FIGURE 6.** Responses of retinal arterioles from wild-type mice to acetylcholine (10^{-5} M) (A) and to the NO donor nitroprusside (10^{-5} M) (B) that had been perfused either with Krebs buffer (vehicle) only or with Krebs buffer containing 0.3% of CHAPS before. Remarkably, acetylcholine produced vasoconstriction after perfusion with CHAPS, whereas vasodilation responses to nitroprusside were retained. Values are expressed as mean ± SE (n = 5 per group; **P < 0.01, CHAPS-treated versus vehicle-treated).

**DISCUSSION**

The major goal of the present study was to identify the muscarinic receptor subtype that mediates vasodilation of murine retinal arterioles to acetylcholine. Since commercially available muscarinic receptor antibodies lack subtype specificity in mice and rats, we used real-time PCR to determine expression of individual muscarinic receptor subtypes in murine retinal arterioles.21–25 Remarkably, we detected mRNA of the M5 receptor only in isolated retinal arterioles. Due to the lack of muscarinic receptor agonists and antagonists with pronounced subtype selectivity, we used M5 receptor-deficient mice to assess the functional relevance of this receptor subtype in retinal arterioles. Although there are some subtype-preferring agonists and antagonists for individual muscarinic receptors (including the M4 receptor) available, it has been shown that their selectivity is only moderate.4 In particular, the M4 receptor shares very similar functional and ligand-binding properties with the M3 receptor, which hampers the differentiation between these two receptor subtypes by classical pharmacologic approaches.26

By modification of a method that has previously been used for measurements of vascular reactivity in the bovine retina, we were able to measure responses of murine retinal arterioles...
by video microscopy with high optic resolution in vitro.20 Using this method, we observed that retinal arterioles from wild-type mice had the ability to dilate in response to acetylcholine. In contrast, deletion of the M3 receptor gene almost completely abolished acetylcholine-induced vasodilation of retinal arterioles. However, responses to the NO donor nitroprusside did not differ between wild-type and M3R−/− mice, suggesting that the lack of M3 receptors did not affect the ability of vascular smooth muscle to relax. The retina of mice and other species was shown to exert substantial acetylcholinesterase activity.25,26 To exclude the possibility that potential differences in retinal acetylcholinesterase activity between wild-type and M3R−/− mice were responsible for the different reactivities of retinal arterioles to acetylcholine, we tested responses of retinal arterioles to the acetylcholine analog carbachol, which is resistant to degradation by acetylcholinesterase. Remarkably, carbachol did not induce significant vasodilation in retinal arterioles from M3R−/− mice, thus excluding the possibility that changes in acetylcholinesterase activity might contribute to the different acetylcholine responses in wild-type and M3R−/− mice. An earlier study suggested that nicotinic acetylcholine receptors may participate in cholinergic vasodilation in canine retinal blood vessels.27 However, in our vascular preparations, vasodilation to acetylcholine was negligible after treatment with the non-subtype-selective muscarinic receptor antagonist atropine, suggesting that acetylcholine-induced vasodilation in murine retinal arterioles is predominantly mediated by muscarinic receptors.

A previous study using electrical stimulation of parasympathetic nerve pathways and intravenous administration of subtype-prefering muscarinic receptor antagonists suggested that endothelial M3 receptors mediate cholinergic vasodilation in the choroid of pigeons.15 Using mice with targeted disruption of the M3 receptor gene, we recently demonstrated that responses of ophthalmic arteries to acetylcholine are also mediated by M3 receptors.16 Thus, the present study together with the previous findings in other ocular vascular preparations indicate that the M3 receptor subtype is the predominant mediator of cholinergic vasodilation within the ocular circulation. Interestingly, murine retinal arterioles seem to differ with regard to their cholinergic vasodilation mechanisms from murine cerebral vessels, where acetylcholine-induced responses were shown to be mediated by the M4 receptor subtype.15 Thus, retinal and cerebral vascular tone may be independently influenced by selective targeting of these two receptor subtypes. It remains to be established, however, whether this difference between cerebral and retinal vessels also exists in humans. Although the M4 receptor has also been proposed to mediate vasodilation in the human brain, there are so far no data regarding the expression and functional role of individual muscarinic receptor subtypes in human retinal vessels.11

Our data suggest that cholinergic vasodilation of murine retinal arterioles is primarily mediated by NO, since blockade of NO synthases by L-NAME almost completely abolished responses to acetylcholine. This finding is consistent with previous studies in bovine and rat retinal arterioles, where responses to acetylcholine were shown to be abolished or impaired after incubation with NO synthase blockers.20,28,29 After endothelial damage with CHAPS, no dilation but vasoconstriction of retinal arterioles to acetylcholine were observed, whereas dilation to the NO donor nitroprusside was retained. This finding indicates that the source of NO needed for cholinergic vasodilation in the retina is the endothelium. A possible explanation for the vasoconstriction responses of retinal arterioles after endothelial damage is that muscarinic receptors are also localized on vascular smooth muscle cells or pericytes and can induce contraction of these cells when activated. Support for this hypothesis comes from studies in isolated bovine retinal arteries, where acetylcholine-induced vasodilation was reversed to contraction when the endothelium was removed, and from experiments in isolated pericytes of the rat retina demonstrating that the muscarinic receptor agonist oxotremorine was able to induce Ca2+−dependent contractions.30,31 These findings suggest that a vasoconstrictor response of cholinergic agents is masked in retinal arterioles when the endothelium is intact.

The physiologic role of acetylcholine in the control of retinal perfusion is unknown at present. In contrast to most other blood vessels, the retinal vasculature lacks autonomic innervation.51,52 Thus, the vessels and the starburst amacrine cells have been proposed to be possible sources of acetylcholine according to their ability to synthesize this substance.33–35 However, whether stimulation of these cells may evoke acetylcholine release in conjunction with muscarinic receptor-mediated vascular responses must still be established. A series of in vivo studies in humans has shown that systemic administration of the non-subtype-selective muscarinic receptor agonist atropine does not affect retinal and choroidal perfusion.36–38 Thus, either pharmacologic blockade of muscarinic receptors can be compensated by other vascular mechanisms to keep ocular blood flow constant or the receptors do not play a major role in regulation of retinal and choroidal perfusion. In contrast, activation of muscarinic receptors with pilocarpine was shown to increase pulsatile ocular blood flow in subjects with ocular hypertension.39 Moreover, various cholinergic substances were reported to increase diameter or blood flow in retinal, choroidal, and retrobulbar vessels of different animal species in vivo.29,40,41 Thus, even if acetylcholine does not play a role in physiologic regulation of ocular blood flow, selective muscarinic receptor agonists may become therapeutically useful to modulate perfusion in the retina and choroid. However, before the M4 receptor may be considered as a therapeutic target to increase retinal perfusion under pathophysiologic conditions, the mechanisms mediating vasoconstriction in retinal arterioles with dysfunctional endothelium need to be determined. This is particularly important because most diseases associated with impaired ocular perfusion, such as diabetic retinopathy and glaucoma, are also associated with endothelial dysfunction.42–45

In conclusion, the data of the present study provide evidence that cholinergic vasodilation of murine retinal arterioles is mediated by endothelial M3 muscarinic acetylcholine receptors, involving activation of NO synthase.

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References
