Nitric Oxide and Choroidal Blood Flow Regulation

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Purpose. Nitric oxide (NO) has been found to be an endothelial-derived relaxing factor mediating the vasodilatation that results from the stimulation of muscarinic endothelial receptors. It also has been identified as a putative neurotransmitter of parasympathetic origin in choroidal perivascular autonomic fibers. The authors investigated a potential role of NO in choroidal blood flow (ChBF) regulation.

Methods. Local ChBF in the tapetal region of 26 anesthetized cats was measured by laser Doppler flowmetry. Cats were infused through the femoral vein with increasing dosages of acetylcholine (ACh); N^{ω}-nitro-L-arginine (NNL-A), a specific inhibitor of NO synthesis; L-arginine; and D-arginine. ChBF and mean arterial pressure (MAP) were continuously recorded.

Results. Infusion of 20 μg/minute ACh induced a 68% increase in ChBF despite a 9% decrease in MAP. Infusion of 16 mg/minute NNL-A attenuated the ACh-induced increase in ChBF by 46% and increased MAP by 40%. Infusion of different dosages of NNL-A without prior administration of ACh caused ChBF to fall below and MAP to rise above baseline in a dose-dependent fashion. Infusion of L-arginine prior to ACh infusion enhanced by 27% the ACh-induced increase in ChBF, whereas D-arginine had no effect on this increase.

Conclusions. These findings suggest the presence of a local vasodilatory cholinergic mechanism in the choroid, inducing the release of NO. They also suggest that release of NO in the choroid may maintain basal blood flow to this tissue. Invest Ophthalmol Vis Sci. 1995;36:925–930.

The vascular smooth muscle relaxation induced by acetylcholine (ACh) and other substances is endothelium dependent and mediated by the release of endothelium-derived relaxing factor (EDRF).[1] Nitric oxide (NO), or a closely related compound, appears to be the EDRF.[2] ACh acts at muscarinic receptors located on vascular endothelial cells[1] and induces the release of NO. Nitric oxide relaxes arteriolar smooth muscles by stimulating the formation of cyclic GMP.[3] Nitric oxide is synthesized from L-arginine by NO synthase (NOS), an enzyme that can be inhibited by several analogues of the amino acid L-arginine, including N^{ω}-nitro-L-arginine (NNL-A). NNL-A inhibits endothelium-dependent vasodilation in the isolated perfused rat mesentery.[4–6] In cats in particular, NNL-A decreases the dilator response of systemic blood vessels to ACh and increases systemic arterial blood pressure. In these animals, a continuous tonic release of endogenous NO also seems to maintain some vascular beds in a dilated state during basal conditions.[4]

The choroidal vasculature is regulated by parasympathetic and sympathetic innervations.[7] Laser Doppler flowmetry indirectly has confirmed both adrenergic and cholinergic receptors in the choroidal vasculature of cats.[8] The neural isomer of NOS has been identified in parasympathetic nerves in the choroid,[9] and a different isomer is likely expressed by endothelial cells in the choroid, indicating that NO might act in this tissue by neural or endothelial mechanisms. Two observations suggest a role for NO in setting basal choroidal blood flow. First, in Beagle dogs, infusion of the NOS inhibitor nitro-L-arginine methylester decreased ChBF, as measured with tracer microspheres.[10] Second, cat choroidal blood vessels dilate in response to ACh,[11,12] a substance known to be a potent stimulus of L-arginine-derived NO in various vascular beds.[13]

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Supported in part by National Eye Institute grants EY03242 and EY05454, a Research to Prevent Blindness Senior Scientific Investigator award (RAS), and The Pennsylvania Lions Sight Conservation and Eye Research Foundation.
Submitted for publication September 6, 1994; accepted November 1, 1994.
Proprietary interest category: N.
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This study in cat choroid investigates the potential role of NO in basal vascular tone and in ACh-induced vasodilatation. The recently developed noninvasive technique of laser Doppler flowmetry was used to measure ChBF.[12]

MATERIALS AND METHODS

Animal Preparation

ChBF was studied in 26 adult cats, each weighing between 2 and 3.5 kg, as described.[12] After premedication with atropine (0.04 mg/kg, subcutaneously), anesthesia was induced with intramuscular ketamine hydrochloride (22 mg/kg) and acepromazine maleate (2 mg/kg). In each cat, a femoral artery and both femoral veins were catheterized, and the animal was ventilated with 21% O_2 and 79% N_2. Anesthesia was maintained with inhalation of enflurane (1.7% to 2.5%), with intravenous pancuronium bromide (loading dose of 0.2 mg/kg, maintenance dose of 0.1 mg/kg per minute) as a muscle relaxant. Arterial blood pressure, tidal CO_2, and heart rate were monitored continuously. Arterial pH, P_{CO_2}, and P_{O_2} were measured intermittently using a blood gas analyzer. Adjustments of the inspired gas mixture, tidal volume, and respiration rate maintained pH $\approx$ 7.4, $P_{CO_2} \approx$ 31 mm Hg, $P_{O_2} \geq$ 90 mm Hg. Rectal temperature was maintained at $\approx$38°C using a heating pad. The pupil of the measured eye was dilated with 1% tropicamide and 10% phenylephrine. The cat was placed prone on a table with the head secure in a stereotactic head holder. A metal ring was sutured to the episcleral limbus and held in a fixed position to prevent eye motion. The cornea was protected from drying by the insertion of a plano soft contact lens and instillation of topical Healon (Pharmacia Ophthalmics, Monrovia, CA). At the conclusion of each experiment, the animal was killed with 5 ml of pentobarbital while still under general anesthesia.

All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Presbyterian Medical Center of Philadelphia Guidelines on Animal Research.

Laser Doppler Flowmetry

Laser Doppler flowmetry measures the flux of red blood cells in a small volume of a vascularized tissue.[12,14] In its application to the measurement of ChBF, a diode laser beam (λ = 0.81 μm) was delivered to the eye through a Topcon fundus camera (model TRC; Tokyo, Japan),[15] modified to include a television camera with monitor and an optical fiber with a nominal aperture of $\approx$450 μm to detect the scattered light in the fundus image plane of the camera. The laser beam was defocused to approximately 500 μm, and the image of the optical fiber aperture was placed over the center of the fundus site illuminated by the laser. The photocurrent generated by the scattered light was analyzed by a blood perfusion monitor (BPM403A, Vasamedic, Minneapolis, MN; or PeriFlux PF3, Perimed, Stockholm, Sweden). Relative flow, F, defined as velocity times volume of red blood cells within the illuminated tissue volume, was recorded. The Doppler shift power spectrum was displayed on an oscilloscope using a spectrum analyzer.

To ascertain that the measured flow represented ChBF in the choriocapillaris, we limited our study to sites meeting the following previously described criteria[11]: (i) the laser beam was aimed at sites in the tapetal region of the fundus, away from visible retinal vessels (intervascular regions); (ii) the Doppler shift power spectrum decayed with increasing frequency, and the frequency at which the power of the Doppler shift power spectrum was 10% of the power at the low frequency end was below 800 Hz; (iii) the decrease in measured flow was less than 5% when the cat breathed 100% O_2 for 4 minutes. Beam location was verified intermittently during the experiments, with appropriate repositioning. Nearly all the sites qualifying as choriocapillaris by the above criteria were more than 2 disk diameters away from the optic disk, either temporally or nasally.

Laser beam alignment was checked before each infusion, and ChBF was continuously monitored before, during, and after each infusion. This procedure was followed for each drug infusion.

Effect of Infusion of ACh on ChBF

In eight cats, ACh solution (1.35 mg in 50 ml of physiological saline) was infused into a femoral vein at rates of 10 and then 20 μg/minutes, with a $\approx$10-minute interval between each rate. Mean arterial pressure was continuously recorded throughout each experiment. The changes in MAP and ChBF were determined from the baseline values and the values when ChBF reached a plateau (see ref. 12, Fig. 4).

Effect of NNL-A Infusion on the ChBF Response to ACh

In six cats, ACh was infused at 20 μg/minutes before and during the infusion of NNL-A. For at least 4 minutes before each increase in the infusion rate, NNL-A (375 mg diluted in 35 ml of 0.05 N HCl normal saline) was infused at increasing rates of 2, 4, 8, and 16 mg/minute. The changes in MAP and ChBF were evaluated from the baseline values and from the plateau values after each NNL-A dose.

Effect of NNL-A Infusion on MAP and ChBF

In six cats, without Ach infusion, NNL-A was infused continuously at increasing rates of 8, 16, and 25.5 mg/

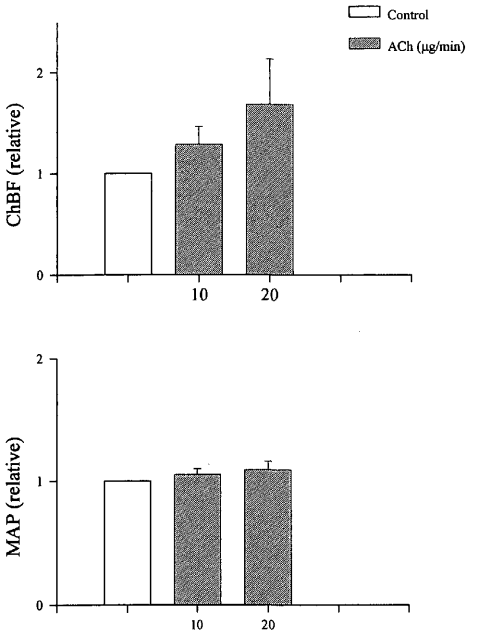

FIGURE 1. Effect of 10- and 20-μg/minute infusions of ACh on choroidal blood flow (ChBF). Error bars are standard deviations (eight cats). The changes in ChBF were determined from the baseline value and the value when ChBF reached a plateau (see Fig. 4).[12] The ChBF and MAP changes were significant at $P < 0.002$ and $P < 0.03$, respectively.

minute, separated by ≈4 minute intervals: The differences between the values of ChBF and MAP before and 4 minutes after each dose of NNL-A infusion were determined.

Effect of L-arginine and D-arginine Infusions on the ACh-induced Change in MAP and ChBF

While ChBF and MAP were continuously measured, ACh was infused at 20 μg/minute for 4 minutes. After this infusion, 375 mg L-arginine diluted in 11.7 ml normal saline was infused in six cats at 16 μg/minute, for a total of 180 mg. ACh was then infused at 20 μg/minute for 4 minutes, starting immediately at the end of the infusion of L-arginine. The percentage changes in ChBF and MAP between the values at baseline and the values when ChBF reached a plateau were determined. The differences in these ACh-induced changes in ChBF and MAP before and after the administration of L-arginine were calculated. In five cats, the protocol was repeated except that D-arginine was substituted for L-arginine.

For all experiments, the change in choroidal vascular resistance (ChVR) was determined from the relationship ChVR = PP/ChBF, where PP = perfusion pressure = MAP − IOP, and IOP is the intraocular pressure. IOP, not measured in these experiments to prevent inducing any corneal irregularities that might interfere with the ChBF readings, was assumed to remain constant at a value of 20 mm Hg.

Statistical Analysis

Percentage changes in the measured parameters were expressed as mean ± SD. Student's paired *t*-test was used, and $P < 0.05$ was considered statistically significant.

RESULTS

The time course of the responses of ChBF and MAP to intravenous infusions of ACh conformed to previously published recordings (data not shown).[11] Despite an initial rapid decrease in MAP, ChBF usually did not decrease, although an occasional transient reduction did occur. More significant, even with MAP below baseline, ChBF returned to baseline usually within 1 minute and reached a plateau in approximately 4 minutes. The increase in ChBF (Fig. 1) and the calculated decrease in ChVR were dosage dependent. With 10 and 20 μg/minute of ACh, despite a small decrease in MAP, ChBF increased by 28% ± 18% and 68% ± 45% ($P < 0.01$), and ChVR decreased by 24% ± 10% and 43% ± 12% ($P < 0.01$), respectively. For the cats used in this experiment, mean basal MAP was 107 ± 25 mm Hg.

Figure 2 shows a recording of MAP and ChBF after

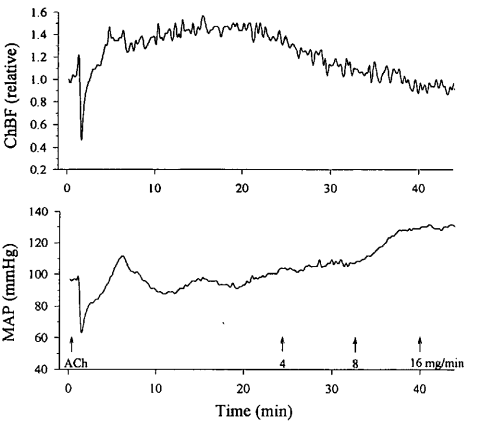

FIGURE 2. Typical recording of MAP and ChBF while 20 μg/minute of ACh was infused and after the administration of N^{ω}-nitro-L-arginine (NNL-A).

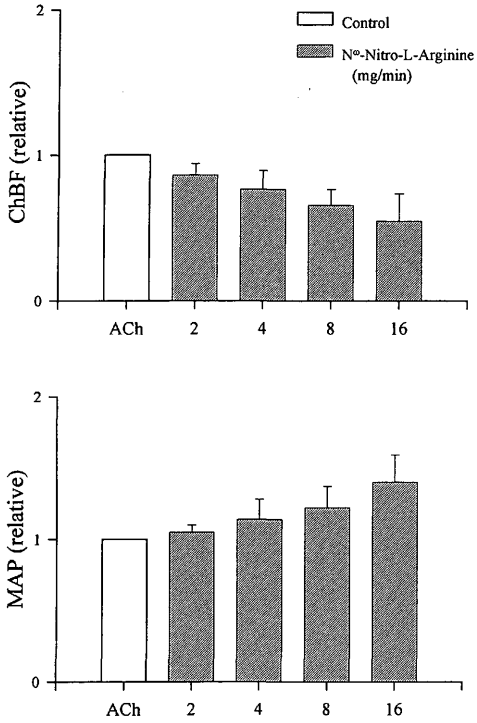

FIGURE 3. Effect of infusing N^{ω}-nitro-L-arginine (NNL-A) on choroidal blood flow, ChBF, and mean arterial pressure (MAP). ChBF decreased in spite of an increase in MAP (six cats). The decreases in ChBF were significant at $P < 0.01$ (2 and 4 mg/minute) and $P < 0.002$ (8 and 16 mg/minute). The increases in MAP were significant at $P < 0.03$.

the administration of NNL-A while ACh was infused at a rate of 20 μg/minute. Mean MAP at baseline was 97 ± 19 mm Hg. As ACh was continuously infused, the administration of NNL-A resulted in a decrease in ChBF in spite of a concomitant increase in MAP. The changes in ChBF, MAP, and ChVR were dosage dependent (Fig. 3). With 16 mg/minute NNL-A, ChBF decreased by 46% ± 19% ($P < 0.002$), MAP increased by 40% ± 19% ($P < 0.03$), and calculated ChVR increased by 200% ± 130% ($P < 0.01$).

Figure 4 shows the effect of various dosages of NNL-A on MAP and ChBF, in the absence of ACh. Baseline MAP for this group of cats was 102 ± 14 mm Hg. The changes in ChBF and MAP were dose dependent. With 25.5 mg/minute NNL-A, ChBF decreased by 50% ± 17%, MAP increased by 21% ± 16%, and ChVR increased significantly by as much as 240% ± 85% ($P < 0.001$).

Infusing L-arginine (Fig. 5) significantly enhanced the ACh-induced increase in ChBF by 27% ± 18% ($P < 0.02$) but had no significant effect on the ACh-induced change in MAP (3% ± 4%). In these cats, mean baseline MAP was 103 ± 12 mm Hg. No significant effect was observed on the ACh-induced changes in MAP and ChBF when D-arginine was administered in the same fashion because these changes before and after D-arginine were −20% ± 8% and −21% ± 6% for MAP, and 37% ± 27% and 38% ± 20% for ChBF.

DISCUSSION

By the response to the intravenous infusion of ACh, the present study indirectly demonstrates that the choroidal vasculature contains receptors for ACh and that activation of these receptors produces a dose-dependent vasodilatation. This finding confirms a previous study in cats in which ACh was infused either intraarterially[11] or intravenously.[12]

We found that the arginine analog NNL-A, a in-

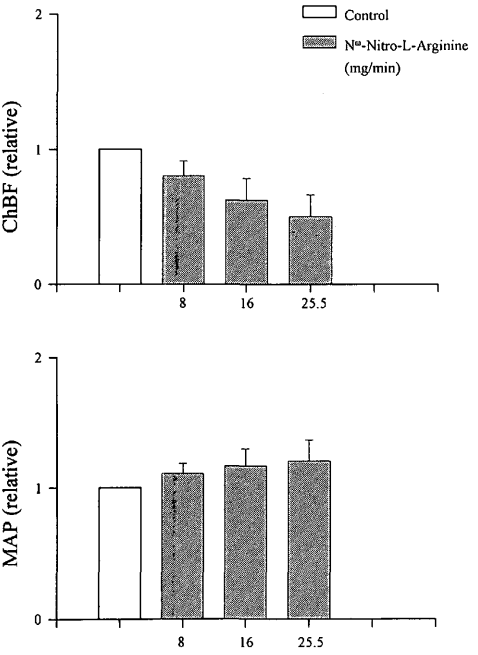

FIGURE 4. N^{ω}-nitro-L-arginine (NNL-A) dose dependence of mean arterial blood pressure (MAP) and choroidal blood flow (ChBF) (six cats). ChBF decreased, whereas MAP increased. The decreases in ChBF were significant at $P < 0.02$ (8 mg/minute) and $P < 0.002$ (16 and 25.5 mg/minute). The increases in MAP were significant at $P < 0.03$.

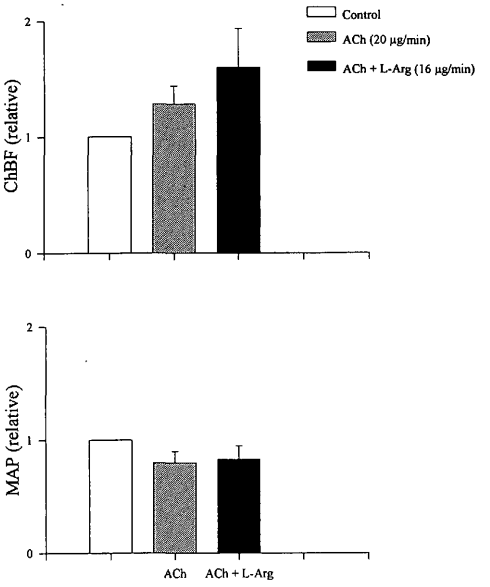

FIGURE 5. Effect of L-arginine (180 mg) on the changes in MAP and ChBF induced by the infusion of 20 μg/minute of Ach (six cats). L-arginine enhanced significantly ($P < 0.02$) the response of ChBF to the infusion of ACh, but not the response of MAP.

hibitor of NO synthesis in vitro and in vivo,[4–6] decreased in a dose-dependent manner the ACh-induced vasodilatation of the choroid. Therefore, these results also suggest a role for NO in the control of choroidal blood flow.

Other findings of this study strongly support the notion that tonic release of NO maintains choroidal vessels in a dilated state under resting physiological conditions. First, NNL-A administration without prior ACh infusion decreased basal ChBF and increased ChVR, despite a dose-dependent increase in MAP. Because the cat choroidal circulation does not autoregulate under normal conditions,[16] this increase in MAP should have produced a parallel increase in ChBF. To the contrary, after NNL-A administration, ChVR increased and ChBF decreased significantly, which suggests a basal vasodilatory state in the choroid that is inhibited by the drug. This finding agrees with results in dogs that showed the infusion of the reversible NOS inhibitor, nitro-L-arginine methylester, also produced a significant decrease in ChBF in spite of an increase in MAP.[13] Second, the administration of L-arginine, a substrate for NOS, enhanced the effect of ACh on ChBF; but D-arginine, which does not inhibit NOS,[17] failed to alter the ACh-induced increase in ChBF. This stereoselectivity is consistent with an enhancement of NOS activity when ACh is infused.

In the present experiments, the direction of the changes in ChBF was always opposite those in MAP. As a result, the changes in ChVR were of greater magnitude than those in ChBF. These changes are consistent with prior results in dogs.[10]

In conclusion, our findings support the presence of local vasodilatory cholinergic receptors in the choroid that induce the release of NO from L-arginine. Based on current concepts, these receptors likely are located on vascular endothelial cells. The basal release of NO in the choroid, at least in part, maintains a vasodilatory tone of these vessels. It is possible that the source for NO is the vascular endothelial cells themselves, which likely contain the endothelial isomer of NOS. Given the localization of the brain isomer on NOS in parasympathetic nerve fibers in the choroid,[9,18] an additional or alternative regulatory mechanism could involve neural elements within the choroid.

Key Words

nitric oxide, choroidal blood flow, cats, laser Doppler flowmetry, acetylcholine, N^{ω}-nitro-L-arginine

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