Role of Nitric Oxide in Maintenance of Basal Anterior Choroidal Blood Flow in Rats

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PURPOSE. The aim of this study was to use laser Doppler flowmetry to measure anterior choroidal blood flow in the anesthetized rat and to determine the role of nitric oxide (NO) in the maintenance of basal ocular blood flow in vivo.

METHODS. By using laser Doppler flowmetry, blood flow from the anterior choroid in pentobarbital-anesthetized rats was measured continuously. Graded single doses (0.03-300 mg/kg) of the nonselective NO synthase inhibitor N^G^-nitro-L-arginine-methyl ester (L-NAME) were administered intravenously to establish dose-response relationships. Other groups of animals were tested with L-NAME after the prior administration of L-arginine, with D-NAME, or with the selective neural NO synthase inhibitor 7-nitroindazole.

RESULTS. Intravenous administration of L-NAME produced a dose-related depression of anterior choroidal blood flow in the 0.3- to 30-mg/kg range. Maximal depression of approximately 60% occurred at the 30-mg/kg dose, peaked at approximately 30 minutes, and lasted throughout the 60-minute experimental period. At 10 mg/kg, L-NAME reduced ocular blood flow by approximately 50%, an effect that was abolished by pretreatment with intravenous L-arginine (300 mg/kg). Both D-NAME (10 mg/kg, intravenously) and 7-nitroindazole (50 mg/kg, intraperitoneally) were inactive with regard to ocular blood flow depression.

CONCLUSIONS. Laser Doppler flowmetry appears to be a useful tool for continuous, online measurement of anterior choroidal blood flow in the rat eye. Results with L-NAME and 7-nitroindazole suggest that local tonic generation of endothelial NO plays an important role in the maintenance of basal anterior choroidal blood flow in this species. (Invest Ophthalmol Vis Sci. 1998;39:559-564)

Nitric oxide (NO) has diverse physiological actions including a pivotal role in blood vessels, in which it is released from endothelial cells to regulate basal blood flow throughout the vascular tree.

In vivo studies demonstrating depression of ocular blood flow by NO synthase inhibitors have been carried out using tracer microsphere techniques in rabbits, dogs, and cats.

The purpose of the present investigation was to establish the feasibility of using laser Doppler flowmetry to measure blood flow on a continuous basis from the surface of the rat eye and to determine quantitatively the effect of inhibition of NO synthase with N^G^-nitro-L-arginine-methyl ester (L-NAME) on anterior choroidal blood flow. Our results demonstrate that this technique is suitable for studying ocular blood flow in rats, and they suggest that tonic release of endothelial NO plays a pivotal role in the maintenance of basal anterior choroidal blood flow in this species.

MATERIALS AND METHODS

General

Adult male Sprague-Dawley rats (300-400 g) were anesthetized with pentobarbital (50 mg/kg injected intraperitoneally). In each animal the trachea, one femoral artery, and both femoral veins were cannulated, and each animal was then positioned in a Kopf rat stereotaxic device to immobilize the head. Body temperature was maintained at approximately 37°C with a heating pad. After the animals were placed on positive artificial ventilation using room air with a small-animal respirator (Harvard Apparatus, Millis, MA), neuromuscular relaxation and anesthesia were maintained with intravenous pancuronium (1-mg bolus dose plus 0.1 mg/kg per minute) and pentobarbital (1.0 mg/kg per minute). Arterial blood pressure was measured from a femoral artery using a blood pressure transducer (P23; Statham Laboratories, Hato Roy, Puerto Rico). Because ocular blood flow was dependent on the perfusion pressure, prepa-
A'-nitro-D-arginine-methyl ester; 7NI, 7-nitroindazole; DMSO, dimethyl sulfoxide.

Anterior segment choroidal blood flow was measured by laser Doppler flowmetry using a Perimed laser Doppler (PF2) flowmeter and a PF103 fiber optic probe.15-17 Basically, this technique exposes a small surface area to coherent laser light generated from helium-neon; the light is reflected from stationary tissue and blood cells, and the moving blood cells produce a Doppler frequency shift that creates Doppler beat frequencies at the photodetector. The computer-processed Doppler beat frequencies are proportional to the total blood cell velocity.18,19 The probe was placed just posterior to the limbus, and care was taken to avoid recording blood flow from the large external limbal blood vessels.20 The total internal diameter of this probe (containing both fiber optic cables) was approximately 1 mm. We speculated that the maximal depth of penetration was approximately 0.5 mm. A drop of mineral oil was used to maintain good optic coupling between the probe and the surface of the eye. Zero ocular blood flow was determined in each preparation after killing the rats at the conclusion of the experiment. It was likely that blood flow signals measured from this site included flows from the anterior uvea and from the anterior, peripheral choroid. For convenience and because the choroidal circulation greatly predominates in the eye, we refer only to anterior choroidal blood flow.

Protocols
To establish dose-response relationships, single intravenous bolus injections of saline or L-NAME (0.03-300 mg/kg) were administered to nine groups of rats. Only one injection was given (during approximately 60 seconds) to each animal. Additional groups of rats were used to study the effects of L-arginine alone, L-arginine with L-NAME, dimethyl sulfoxide (DMSO), and 7-nitroindazole. In a separate series of experiments, parietal cerebral cortical blood flow measurements were made through a cranial window to test the effectiveness of 7-nitroindazole in reducing cerebral blood flow.

Drugs
Pancuronium bromide was purchased from Sigma Chemical (St. Louis, MO). The L- and D-isomers of A'-nitro-L-arginine-methyl ester, D-NAME, N5-nitro-L-arginine-methyl ester; 7NI, 7-nitroindazole; DMSO, dimethyl sulfoxide.

**Table 1. Comparisons (30-Minute Time Points) of Anterior ChBF Expressed as Percentage of Initial Control Levels, MSAP, and HR**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ChBF Percentage of Initial Control Before</th>
<th>ChBF Percentage of Initial Control After</th>
<th>MSAP (mm Hg) Before</th>
<th>MSAP (mm Hg) After</th>
<th>HR (beats/min) Before</th>
<th>HR (beats/min) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>93 ± 4</td>
<td>114 ± 9</td>
<td>424 ± 15</td>
<td>420 ± 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NAME (10 mg/kg)</td>
<td>54 ± 7†</td>
<td>114 ± 7</td>
<td>438 ± 15</td>
<td>383 ± 15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine (300 mg/kg)</td>
<td>89 ± 6*</td>
<td>121 ± 7</td>
<td>420 ± 14</td>
<td>394 ± 20*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine (300 mg/kg) + L-NAME (10 mg/kg)</td>
<td>95 ± 6</td>
<td>92 ± 13</td>
<td>381 ± 30</td>
<td>334 ± 25*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-NAME (10 mg/kg)</td>
<td>91 ± 5</td>
<td>111 ± 14</td>
<td>398 ± 38</td>
<td>365 ± 44*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7NI Vehicle (DMSO)</td>
<td>95 ± 4</td>
<td>133 ± 7</td>
<td>506 ± 18</td>
<td>491 ± 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7NI (50 mg/kg)</td>
<td>93 ± 6</td>
<td>105 ± 9</td>
<td>474 ± 20</td>
<td>467 ± 14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± SEM. Measurements taken before and 30 minutes after drug or vehicle administration.

*P < 0.05.
†P < 0.01.

Table 1: Comparisons (30-Minute Time Points) of Anterior ChBF Expressed as Percentage of Initial Control Levels, MSAP, and HR

**Statistical Analysis**
Results are expressed as mean ± SEM. Multiple group responses to drug dosages were compared with two-way analysis of variance (ANOVA) and by Dunnet’s test. Responses to saline were used for the control group. Comparison of single time points within groups (for example, cardiovascular data before and after treatment) was by paired Student’s t-test. P < 0.05 was considered to indicate statistical significance.

**RESULTS**

**Controls and Dose-Response Relationships**
The first series of experiments was designed to determine the feasibility of using laser Doppler flowmetry to measure anterior choroidal blood flow in the rat eye, to demonstrate the stability of blood flow measurements with time, and to establish the effectiveness and optimal dose of L-NAME needed to significantly depress anterior choroidal blood flow in this species.

In eight control animals (intravenous administration of physiological saline), there were no significant alterations of
Nitric Oxide and Rat Anterior Choroidal Blood Flow

A Nitric Oxide and Rat Anterior Choroidal Blood Flow

B

FIGURE 1. Effects of intravenous (A) saline or (B) Nω-nitro-L-arginine-methyl ester (ω-NAME) (10 mg/kg) on anterior choroidal blood flow and systemic arterial blood pressure in two pentobarbital-anesthetized rats. Control responses were taken immediately before administration, and effects of saline or ω-NAME were taken after 30 minutes. Note the stability of the control responses and the effect of ω-NAME simultaneously to elevate systemic arterial blood pressure and to decrease choroidal blood flow.

Mechanistic Studies

Additional groups of animals were used to define the underlying mechanisms involved with ω-NAME-induced depression of anterior choroidal blood flow. To establish that the effect of ω-NAME was specific to the inhibition of ocular NO synthase, one group of animals was administered the inactive isomer ω-D-NAME, and a second group of rats was pretreated intravenously with l-arginine before ω-NAME administration. As shown in Table 1 (30 minutes after administration), intravenous ω-D-NAME (10 mg/kg) was inactive with regard to ocular blood flow reduction, as was ω-NAME (10 mg/kg) administered to animals pretreated with l-arginine (300 mg/kg).

In an additional group of animals, the selective neuronal NO synthase inhibitor, 7-nitroindazole (50 mg/kg given intraperitoneally), also was inactive in causing significant reduction of ocular blood flow (Table 1). The 7-nitroindazole vehicle, DMSO, was without effect on any of the measured parameters in five DMSO-treated rats (Table 1). In a separate group of rats (data not shown), intraperitoneal administration of 7-nitroindazole (30 mg/kg) produced a depression of 35 ± 7% in cerebral blood flow (n = 7, P < 0.05). Cardiovascular changes (at 30 minutes) for ω-NAME (10 mg/kg) and the other experimental groups are summarized in Table 1.

DISCUSSION

Laser Doppler flowmetry is an accepted method for continuous measurement of localized blood flow in organs such as the skin, kidney, and nerves, which are difficult to study by direct blood flow measurement techniques.18 This technology has been used to measure retinal and posterior choroidal blood flow in cats and rabbits,19,21 and anterior choroidal blood flow in cats.15,16,22,23 and in the pigeon.24 In the present study, we have extended this technique of surface laser Doppler blood flow measurements to the rat eye.

In these experiments, we were able to make continuous online measurements of blood flow in the anterior choroid of the anesthetized rat that were stable for the duration of the experiment. The main limitations inherent with laser Doppler flowmetry include restriction to a small measurement area,
Nitric oxide is found throughout the vascular system including the vasculature of the eye, particularly the choroidal blood vessels. The rat choroid was among the first vascular beds in which the presence of NO synthase was demonstrated. Extensive NO synthase immunoreactive nerve fibers are localized around blood vessels of the rat limbus and choroid with less staining in the iris and ciliary body. Others have demonstrated diaphorase-positive NO synthase-containing nerve fibers surrounding arteries and arterioles of the choroidal stroma of a variety of species including rats, rabbits, and humans.

Inhibitors of NO synthase produce contractions of isolated ocular blood vessels, including porcine ophthalmic and ciliary arteries. They also have been shown to block neurogenic lack of calibration in meaningful units of blood flow, and uncertainty concerning the precise volume of tissue from which blood flow is measured. As mentioned previously, anterior uveal blood flow likely contributes to the anterior choroidal blood flow measurement. The reduction of signal by tissue pigment that was problematic in the cat anterior choroid is not a limitation of this technique in the albino rat.

![Figure 2](image1.png)

**Figure 2.** Composite representations of time course of intravenously administered L^-nitro-L-arginine-methyl ester (L-NAME; 0.03-30 mg/kg)-induced (A) depression of anterior choroidal blood flow as measured from eyes of anesthetized rats using laser Doppler flowmetry and (B) elevations of mean systemic arterial blood pressure in the same animals. Each line represents mean responses (percentage of initial control values) from six to eight animals taken at the times indicated. Values are expressed as mean ± SEM (statistical evaluations are shown in Fig. 3). Maximal increases in systemic arterial blood pressure were seen within 5 to 10 minutes, whereas maximal depression of anterior choroidal blood flow was delayed for approximately 30 minutes.

![Figure 3](image2.png)

**Figure 3.** Peak effects of intravenous administration of L^-nitro-L-arginine-methyl ester (L-NAME; 0.1-300 mg/kg) on (A) anterior choroidal blood flow and (B) mean systemic arterial blood pressure in seven groups of pentobarbital-anesthetized rats. Each bar height represents mean responses; vertical bars indicate SEM for seven to eight rats per group. Choroidal blood flow values were taken 60 minutes after L-NAME administration. Systemic blood pressure levels represent peak levels seen within the first 10 to 15 minutes of L-NAME administration. *P < 0.05; **P < 0.01.
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In contrast to the present results in rats, two groups of investigators using microsphere tracers failed to demonstrate a statistically significant depression of choroidal blood flow in the cat. A high degree of variability between animals may account for the lack of statistical significance in these cat studies because a definite trend toward choroidal vasoconstriction was apparent in their results, with a mean depression of choroidal blood flow of approximately 30% and 48%, respectively. It is of note that in one study, the cats were initially treated with indomethacin, which may have contributed to an attenuated response to L-NAME.

A unique aspect of the present study was the establishment of dose–response relationships for L-NAME with the maximal effect for anterior choroidal vasoconstriction observed at the 10- to 30-mg/kg dose range. Maximal increases in blood pressure occurred 10 to 15 minutes after injection and were observed at doses in excess of 30 mg/kg. These results are in accordance with other studies establishing dose relationships for L-NAME in causing elevation of arterial blood pressure in anesthetized rats with regard to dose range and extent of vasoconstriction. A similar dose range was required to increase hindquarter perfusion pressure in pentobarbital-anesthetized cats. Because ocular blood flow has been observed to decrease in the presence of large elevations in systemic blood pressure, it is suggested that the true extent of potential ocular vasoconstriction caused by NO synthase inhibition is even greater than reported.

Two lines of evidence confirmed that the ocular vasoconstrictor effect of L-NAME is caused by inhibition of NO synthase. First, the inactive stereoisomer D-NAME was without significant effect when administered at the same dose (10 mg/kg), whereas L-NAME produced dramatic anterior choroidal blood flow depression. Second, pretreatment with L-arginine largely prevented the ocular vasoconstrictive action of subsequent L-NAME administration. Together, these observations support the conclusion that L-NAME acts by inhibition of endogenous NO synthase and not by some nonspecific mechanism.

Evidence in the present study for the endothelial origin of NO comes from experiments using the selective inhibitor of neuronal NO synthase, 7-nitroindazole. Systemic administration of 7-nitroindazole (50 mg/kg) did not cause depression of anterior choroidal blood flow nor did it cause an increase in systemic arterial blood pressure. In the pigeon, this dose of 7-nitroindazole did not alter resting anterior choroidal blood flow or systemic arterial blood pressure. It did, however, dramatically block neurally evoked vasodilator responses elicited by electrical stimulation of the pigeon Edinger-Westphal nucleus. In the present study, a smaller dose of 7-nitroindazole (30 mg/kg) produced a significant reduction of basal blood flow in the parietal cerebral cortex.

In conclusion, the present investigation demonstrates the usefulness of the laser Doppler flowmetry technique for continuous measurement of anterior choroidal blood flow at the surface of the rat eye. Inhibition of NO synthase with L-NAME produced a dose-dependent reduction in ocular blood flow, whereas the selective inhibitor of neuronal NO synthase, 7-nitroindazole, was without significant effect. Taken together, these results suggest that tonic synthesis and release of endothelial-derived NO provides ocular vasodilator tone and plays a pivotal role in maintaining resting anterior choroidal blood flow in the rat eye.
Acknowledgment

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References