Effect of Nitric Oxide Synthase Inhibitor on Optic Nerve Head Circulation in Conscious Rabbits

Tetsuya Sugiyama, Hidehiro Oku, Shoichiro Ikari, and Tsunehiko Ikeda

PURPOSE. To study the effect of a nitric oxide synthase inhibitor on tissue circulation in the optic nerve head (ONH) of conscious rabbits.

METHODS. N^G-nitro-l-arginine methyl ester (l-NAME) (1, 10, or 100 mg/kg), d-NAME (10 mg/kg), or physiological saline was administered intravenously to albino rabbits. A quantitative index of blood velocity, the normalized blur (NB), was measured in the ONH by laser speckle tissue circulation analyzer. The intraocular pressure (IOP) and blood pressure (BP) were also measured. l-arginine (10 mg/kg) was intravenously administered 20 minutes after l-NAME (10 mg/kg) injection. Acetylcholine (ACh; 10 µg/kg per minute) was infused for 15 minutes, with or without pretreatment of l-NAME (1 mg/kg).

RESULTS. l-NAME induced a continuous decrease of the NB in a dose-dependent manner, but d-NAME caused no significant change. At 100 mg/kg, l-NAME significantly increased the IOP, mean BP, and ocular perfusion pressure, but the other doses caused no significant changes. When l-arginine was administered after l-NAME injection, the NB returned to its initial level and remained there. Pretreatment with l-NAME inhibited the increase of NB induced by ACh.

CONCLUSIONS. These results indicate that nitric oxide regulates basal tissue circulation in the ONH of conscious rabbits and suggest that ACh increases the circulation by promoting nitric oxide synthesis. (Invest Ophthalmol Vis Sci. 2000;41:1149–1152)

Nitric oxide (NO) has been identified as an endothelium-derived relaxing factor, and it has been found to play an important role in the regulation of local blood flow. An NO synthase (NOS) inhibitor has been reported to suppress the relaxation of isolated porcine ophthalmic arteries as well as the dilatation of canine retinal arteries elicited by bradykinin, acetylcholine (ACh), or substance P.1,2 A study using laser Doppler flowmetry in anesthetized cats has suggested the presence of a local vasodilatory cholinergic mechanism in the choroid, involving the release of NO, which may maintain basal blood flow to this tissue.3 We previously reported that intravitreal injection of an NO donor increased the optic nerve head (ONH) blood flow when evaluated by the hydrogen gas clearance method.4 Neufeld et al.5 reported that the three isoforms of NOS were apparently increased in the ONH of patients with primary open-angle glaucoma and that an increase of NOS-3 in the vascular endothelium may be neuroprotective by causing vasodilation and promoting tissue blood flow. Buerk et al.6 and Kondo et al.7 concluded that NO release may mediate much of the vasodilating effect of flicker in cats and may play a role in maintaining normal vascular tone in the ONH. NOS was also found in the ciliary muscle, the trabecular meshwork, and the canal of Schlemm, and its activity may be decreased in the eyes of patients with primary open-angle glaucoma.8,9 Intravitreal application of an NO donor reduced the intraocular pressure (IOP) in albino rabbits.10 These findings suggest that NO may also be involved in the regulation of IOP.

In the present study, we tried to clarify the effect of NO on ONH circulation in conscious rabbits, by using the laser speckle method and intravenous administration of an NOS inhibitor alone or combined with l- or d-arginine or ACh.

MATERIALS AND METHODS

Animals

Male albino rabbits weighing 2.7 to 3.4 kg were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). They were housed in an air-conditioned room (22 ± 1°C with 66% ± 3% humidity) with a 12-hour light–dark diurnal cycle and were given food and water ad libitum. They were handled in accordance with the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research.

Drugs Used

N^G-nitro-l-arginine methyl ester (l-NAME) and N^G-nitro-d-arginine methyl ester (d-NAME) were purchased from Sigma (St. Louis, MO). l- or d-arginine and ACh were purchased from Wako (Osaka, Japan). Each was dissolved in physiological saline (Otsuka, Tokyo, Japan).

Measurement of ONH Blood Velocity, IOP, and Blood Pressure

The blood velocity in the ONH was measured with a laser speckle tissue circulation analyzer. The details of this apparatus were previously reported by Tamaki et al.11 Scattered laser
light is projected onto the image sensor, where the laser speckle pattern appears. The normalized blur (NB) obtained with this apparatus is equivalent to a quantitative index of the blurring of a speckle pattern and is an indicator of tissue blood velocity. The relative change of the NB shows a strong correlation with the change in the ONH tissue blood flow when measured by the hydrogen gas clearance method, suggesting that the change of NB is indicative of the change in blood flow. Rabbits were placed in holding boxes, and the measurements described were obtained in animals under local anesthesia with a drop of 0.4% oxybuprocaine hydrochloride (Benoxil; Santen, Osaka, Japan).

For measurement of ONH blood velocity, the average NB over an area of \(0.42 \times 0.42\) mm of the ONH free of surface vessels was measured in a randomly selected eye after mydriasis with a drop of 0.4% tropicamide (Mydrin M; Santen). It takes 0.18 seconds to record 98 scans and obtain one NB value. The NB at each time was calculated as the average of five successive measurements.

The IOP was measured using a calibrated pneumatonometer (Alcon, Tokyo, Japan) in the eye contralateral to that used for blood velocity measurement. One of the auricular arteries was cannulated with polyethylene tubes (SP28; Natume, Tokyo, Japan) in rabbits under local anesthesia with 2% lidocaine (Xylocaine spray; Fujisawa, Tokyo, Japan) for monitoring mean arterial blood pressure (BP). Mean arterial BP was calculated by \([\text{diastolic BP } + \frac{1}{3}(\text{systolic BP } - \text{diastolic BP})]\).

**Effect of NOS Inhibitor**

After establishing the baseline values of NB, IOP, and BP, 1 ml \(L\)-NAME (1, 10, or 100 mg/kg), \(d\)-NAME (10 mg/kg), or physiological saline (control) was injected intravenously into an auricular vein. In another group of rabbits, 1 ml \(L\)-NAME (1 mg/kg) was injected intravenously 5 minutes before the infusion of \(ACh\). The NB, IOP, and BP were measured every 15 minutes for 90 minutes. Each group contained six rabbits.

**Effect of \(ACh\)**

After establishing the baseline values of NB, IOP, and BP, \(ACh\) (10 \(\mu g/kg\) per minute) or physiological saline was infused for 15 minutes into an auricular vein. In another group of rabbits, 1 ml \(L\)-NAME (1 mg/kg) was injected intravenously 5 minutes before the infusion of \(ACh\). The NB, IOP, and BP were measured every 15 minutes for 90 minutes. Each group contained six rabbits.

**Statistical Analysis**

Data are expressed as the means ± SE. Statistical analysis was performed by two-way analysis of variance (ANOVA) for repeated measurements. If statistically significant difference was detected, further assessment was performed by one-way ANOVA followed by Dunnett’s test. A difference was considered significant if \(P < 0.05\).

**RESULTS**

**Effect of NOS Inhibitor**

Baseline values (mean ± SE) of mean BP in \(L\)-NAME (1, 10, and 100 mg/kg), \(d\)-NAME (10 mg/kg) and control groups were 97.5 ± 5.1, 105.2 ± 7.6, 98.1 ± 5.4, 94.7 ± 5.1, and 104.3 ± 6.5 mm Hg, respectively. Baseline values (mean ± SE) of IOP in the same groups were 17.8 ± 0.8, 18.7 ± 1.1, 18.3 ± 0.8, 16.7 ± 0.9, and 18.8 ± 1.3 mm Hg, respectively. There were no significant differences among the groups in these baseline values.

The effects of \(L\)-NAME injection on the NB, IOP, and mean BP are shown in Figures 1, 2, and 3, respectively. Two-way ANOVA for repeated measurements showed significant differences between the control and \(L\)-NAME groups in each parameter.

**Effect of \(ACh\)**

After establishing the baseline values of NB, IOP, and BP, \(ACh\) (10 \(\mu g/kg\) per minute) or physiological saline was infused for 15 minutes into an auricular vein. In another group of rabbits, 1 ml \(L\)-NAME (1 mg/kg) was injected intravenously 5 minutes before the infusion of \(ACh\). The NB, IOP, and BP were measured every 15 minutes for 90 minutes. Each group contained six rabbits.

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Data are expressed as the means ± SE. Statistical analysis was performed by two-way analysis of variance (ANOVA) for repeated measurements. If statistically significant difference was detected, further assessment was performed by one-way ANOVA followed by Dunnett’s test. A difference was considered significant if \(P < 0.05\).

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**Effect of NOS Inhibitor**

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The effects of \(L\)-NAME injection on the NB, IOP, and mean BP are shown in Figures 1, 2, and 3, respectively. Two-way ANOVA for repeated measurements showed significant differences between the control and \(L\)-NAME groups in each param-
eter. One-way ANOVA followed by Dunnett’s test showed that L-NAME (10 and 100 mg/kg) significantly decreased the NB compared with that of the control group in a dose-dependent manner. In one-way ANOVA followed by Dunnett’s test, L-NAME (100 mg/kg) also significantly increased the IOP at 45 and 60 minutes and the mean BP at 15 minutes compared with the control group, whereas there were no significant differences between the control and D-NAME (10 mg/kg) groups.

Changes of ocular perfusion pressure which was calculated as mean BP minus IOP are shown in Table 1. There were no significant differences among the groups in the pretreatment values. Two-way ANOVA for repeated measurements showed significant differences among these groups. One-way ANOVA followed by Dunnett’s test showed that L-NAME at 100 mg/kg significantly increased the ocular perfusion pressure at 15 minutes compared with the control group, whereas it did not change significantly in other groups.

The effect of the additional injection of L-arginine or D-arginine on the NB is shown in Figure 4. The NB was decreased by the injection of L-NAME (10 mg/kg), but after the injection of L-arginine (10 mg/kg), it returned to the initial level and was maintained for 120 minutes. Two-way ANOVA for repeated measurements showed significant differences between the control and L-arginine groups. According to one-way ANOVA followed by Dunnett’s test, there were significant differences between the control and L-arginine groups at 45, 60, and 90 minutes, but there were no significant differences between the control and D-arginine groups.

**Effect of ACh**

The effect of ACh on the NB is shown in Figure 5. Two-way ANOVA for repeated measurements showed that there were significant differences between the control and ACh groups, and between the ACh and L-NAME + ACh groups. According to one-way ANOVA followed by Dunnett’s test, ACh caused a significant increase of the NB at 15 minutes compared with the control group, but there were no significant differences between the control and L-NAME + ACh groups.

**DISCUSSION**

In the present study, the effect of an NOS inhibitor on tissue circulation in the ONH of conscious rabbits was determined

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**Table 1. Changes of Ocular Perfusion Pressure**

<table>
<thead>
<tr>
<th>NOS Inhibitor</th>
<th>Dose</th>
<th>Before</th>
<th>15 Minutes</th>
<th>30 Minutes</th>
<th>45 Minutes</th>
<th>60 Minutes</th>
<th>90 Minutes</th>
<th>120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-NAME</td>
<td>1 mg/kg</td>
<td>80.5 ± 5.4</td>
<td>89.3 ± 7.0</td>
<td>82.1 ± 5.7</td>
<td>85.6 ± 4.3</td>
<td>81.7 ± 3.3</td>
<td>79.9 ± 2.5</td>
<td>79.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>82.3 ± 4.1</td>
<td>90.4 ± 3.6</td>
<td>87.5 ± 4.0</td>
<td>83.8 ± 3.8</td>
<td>81.8 ± 4.5</td>
<td>83.7 ± 4.4</td>
<td>81.3 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>82.5 ± 5.3</td>
<td>101.0 ± 5.4*</td>
<td>96.0 ± 6.0</td>
<td>86.8 ± 8.1</td>
<td>85.3 ± 6.0</td>
<td>85.1 ± 5.1</td>
<td>79.5 ± 5.8</td>
</tr>
<tr>
<td>d-NAME</td>
<td>10 mg/kg</td>
<td>82.6 ± 2.5</td>
<td>80.8 ± 3.2</td>
<td>81.1 ± 2.8</td>
<td>81.8 ± 4.2</td>
<td>81.2 ± 2.7</td>
<td>83.1 ± 4.3</td>
<td>85.3 ± 3.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>85.7 ± 4.4</td>
<td>83.9 ± 4.7</td>
<td>83.6 ± 4.6</td>
<td>82.8 ± 4.6</td>
<td>82.5 ± 5.1</td>
<td>84.0 ± 3.7</td>
<td>81.1 ± 5.1</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE millimeters of mercury for six rabbits. There was a significant difference between these groups (two-way ANOVA for repeated measurements).

* Significantly different from the control group (P < 0.05; Dunnett’s test).
using the laser speckle method, which was first developed by Fercher and Briers.\textsuperscript{13} This is a noncontact method for two-dimensional measurement of tissue circulation in the ocular fundus that was recently developed in Japan.\textsuperscript{11,14–16} The NB in the ONH obtained by this method is not only indicative of tissue blood velocity but also of tissue blood flow under specific conditions.\textsuperscript{12}

There have been previous reports on the effect of NOS inhibitors on the ONH circulation by Buerk et al.,\textsuperscript{5} Harino et al.,\textsuperscript{17} and Kondo et al.,\textsuperscript{7} but they used other methods to determine ONH blood flow (Buerk et al., Harino et al.: laser Doppler flowmetry, and Kondo et al.: microspheres). They also obtained results indicating that the vascular tone in the ONH was affected by NO, although their methods did not allow blood flow measurement over such a long time as in our study and could not be applied to conscious animals. In contrast, our method can directly detect changes under more physiological conditions and in more detail.

Systemic administration of \textit{L}-NAME induced a dose-dependent and persistent decrease of the NB in the ONH, whereas \textit{D}-NAME did not change it. Furthermore, the NB value recovered after the administration of \textit{L}-arginine with \textit{L}-NAME. These results indicate that the ONH circulation was reduced by competitive inhibition of NO synthesis. In other words, NO seems to be essential for maintaining basal ONH circulation. Mean BP and IOP increased only after injection of \textit{L}-NAME at 100 mg/kg. The ocular perfusion pressure also increased significantly only at that dose at 15 minutes and was unchanged at other doses. ONH circulation was reduced although the ocular perfusion pressure was increased or unchanged after systemic administration of an NOS inhibitor, suggesting that NO played an important role in regulating the vascular resistance of the ONH.

The increase of the NB in the ONH during the infusion of ACh was inhibited by pretreatment with \textit{L}-NAME. ACh appeared to increase the ONH circulation by promoting NO synthesis. Since the study by Furchgott and Zawadzki\textsuperscript{18} there have been various reports indicating that ACh dilates blood vessels by releasing NO. Our results confirm that this also occurred in the ONH circulation, at least in rabbits.

The present study suggests that NO regulates basal tissue circulation in the ONH and that ACh may increase the circulation by promoting NO synthesis in conscious rabbits.

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References