Time Course of the Change in Optic Nerve Head Circulation after an Acute Increase in Intraocular Pressure

Jun Takayama, Atsuo Tomidokoro, Kiyoshi Isbii, Yasubiro Tamaki, Yasubiro Fukaya, Tomokazu Hosokawa, and Makoto Araie

PURPOSE. To investigate the time course of changes in optic nerve head (ONH) circulation after an acute increase in intraocular pressure (IOP), by using the laser speckle method, and to evaluate the effects of a calcium antagonist, the nitric oxide synthetase inhibitor, indomethacin, or sympathetic nerve amputation on the response in ONH circulation after an acute increase in IOP.

METHODS. In rabbits, the normalized blurred (NB) level, a quantitative index of tissue blood velocity in the ONH, was monitored for 60 minutes after an increase in IOP from 20 mm Hg to 40 or 50, or 60 mm Hg and for 25 seconds after increase in IOP from 20 mm Hg to 50 or 60 mm Hg with high time resolution. The effects of systemic administration of 1 μg/kg per hour nilvadipine (a calcium antagonist), 30 mg/kg N-nitro-l-arginine (NNAME), or 5 mg/kg indomethacin, or those of sympathetic nerve amputation on the time course of the changes in NB were studied.

RESULTS. NB showed a quick recovery within several seconds after increase in IOP to 40 or 50 mm Hg, whereas no or little recovery occurred after an increase to 60 mm Hg. The nilvadipine treatment significantly increased NB at IOP of 20 mm Hg (baseline NB, P = 0.045) and apparently impaired the recovery of NB after the increase in IOP. After NNAME administration, baseline NB significantly decreased (P = 0.028), and the NB recovery time was slightly but significantly prolonged (P = 0.012). Indomethacin showed no effects on baseline NB or NB recovery. Sympathetic nerve amputation increased baseline NB (P = 0.027), but did not influence NB recovery.

CONCLUSIONS. The current results showed a quick recovery response in the ONH circulation after an acute increase in IOP in rabbits. A calcium antagonist impaired the response. Production of nitric oxide or prostaglandins or the sympathetic nervous system is probably not mainly responsible for the reaction. (Invest Ophthalmol Vis Sci. 2003;44:3977–3985) DOI:10.1167/iovs.03-0024

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It is suggested that blood flow in the retina and optic nerve head (ONH) is stably maintained despite certain changes in ocular perfusion pressure (OPP), and this ability is recognized as a function of autoregulation.1−10 Because vascular insufficiency, including inadequate response to changes in perfusion pressure in the ONH, may play a role in the pathogenesis of glaucoma11−15 and acute ischemic optic neuropathy,16,17 it is of clinical importance to investigate how ONH circulation reacts to the changes in OPP.

Previous studies have mainly focused on the presence of autoregulation or its functional range of OPP. Because most of the studies evaluated the changes in ocular blood flow after certain time intervals (at least 20 minutes) after changes in OPP,1,3,6,7 the time course of circulatory response has not been fully investigated. In recent years, using laser Doppler flowmetry, Riva et al.9 continuously monitored relative changes in blood flow in the cat ONH during stepwise or continuous elevations of intraocular pressure (IOP). Although their results suggest a quick recovery of blood flow within 1 minute after elevation of IOP up to 50 mm Hg, the flow change during the first minute, in which the blood flow was reduced and then recovered, was not recorded. In humans, laser Doppler flowmetry was also used to assess autoregulation in the ONH after stepwise elevations10 or continuous increase11 of IOP by scleral suction cup, and it was suggested that restoration of ONH circulation after increase of IOP (i.e., OPP decrease) is achieved very quickly. Our knowledge about the time course of changes in the ONH circulation just after the IOP alteration, however, is limited, and physiological or pharmacological properties included in it have not been investigated.

The laser speckle method has been developed recently for noninvasive assessment of tissue circulation in living eyes and gives a quantitative index of blood flow velocity and the normalized blurred NB index, which also has been confirmed to correlate well linearly with the blood flow determined with microsphere technique in the iris18 choroid,19 and retina20 and the blood flow determined with the hydrogen gas clearance method in the ONH.21,22 Using this method, continuous monitoring of ONH circulation can be obtained with a high time resolution.23 In this study, we investigated the time course of changes in the ONH circulation after an acute increase in IOP with a high-time-resolution analysis by the laser speckle method. Further, we studied the effects of a calcium antagonist, the nitric oxide synthetase (NOS) inhibitor, indomethacin, and sympathetic nerve amputation on the time course of the change.

METHODS

Animals

In this series of experiments, Japanese albino rabbits weighing 2.0 to 2.5 kg were used and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. After induction of general anesthesia (0.9–1.1 g/kg urethane, intravenously),
the femoral artery was cannulated for monitoring of blood pressure, pulse rate, Po2, Pco2, and pH of the arterial blood. The mean femoral arterial blood pressure (FABPav in mm Hg) was calculated as

\[
\text{FABP}_{av} = \frac{1}{5} (\text{FABP}_{p} + \text{FABP}_{d} + \text{FABP}_{a} + \text{FABP}_{s} + \text{FABP}_{v})
\]

where FABPp and FABPd were the diastolic and systolic femoral arterial blood pressures, respectively. OPP (in mm Hg) was calculated as

\[
\text{OPP} = \text{FABP}_{av} - \text{IOP} - 14
\]

where −14 was the compensator for the discrepancy in pressures between the femoral artery and the ophthalmic artery in a prone positioned rabbit.23,24 The animal was placed in a stereotaxic device equipped with a heating pad, and the body temperature was monitored rectally. In both eyes, the pupil was dilated with 1 drop of 0.4% tropicamide at least 20 minutes before the experiment began. Rabbits that showed abnormal systemic parameters26 after general anesthesia were excluded from the experiments.

**Evaluation of ONH Circulation**

Circulation in the ONH was evaluated using the laser speckle method, details of which have been described previously26–28 and are briefly summarized herein. An apparatus used for the measurements included a fundus camera with a diode laser (wavelength: 808 nm; power: 2 mW), an image sensor, and a personal computer. The laser beam was focused on the surface of the ONH, which was illuminated by a halogen lamp. The scattered light was imaged on an image sensor of 100 × 100 pixels, corresponding to a field of 0.62 × 0.62 mm² in the rabbit fundus, where the speckle pattern appeared. The difference between an average speckle intensity and the speckle intensity of successive scans was calculated. The ratio of the average speckle intensity to this difference was defined as normalized blur (NB). The average NB level in the most widely available rectangular area free of visible vessels in the ONH was calculated and termed NBONH. An NBav measurement took 0.125 second, and successive results for 1 second were averaged and referred to as NBONH.

Blood flow rate in the ONH was determined with a hydrogen clearance flowmeter (RBF-222; Biomedical Science, Kanazawa, Japan). A hydrogen needle electrode (diameter: 0.1 mm) was inserted through the vitreous body into the lower portion of the ONH (depth: approximately 0.7 mm), guided by viewing with a vitrectomy lens. A reference electrode was fixed in the subcutaneous tissue of the head. Capillary blood flow was calculated with hydrogen density half-life, after the inhalation of 10% hydrogen gas by mask at 0.5 L/min for 5 minutes.21,22 After this experiment, some of the rabbits were killed immediately, and the eyes were enucleated to evaluate the histopathology around the ONH in which the hydrogen needle was inserted.

**Correlation between NBONH and ONH Blood Flow Measured by the Hydrogen Gas Clearance Method**

A randomly chosen eye of each rabbit (n = 20) was prepared for ONH blood flow measurement by the hydrogen gas clearance method, and two 25-gauge needles were inserted into the anterior chamber from the limbus. The connecting tube from one of the needles was branched through a turncock into two reservoirs of commercially available artificial aqueous (Opeguard MA; Senju Pharmaceutical Co., Osaka, Japan), which were mounted at different heights. By alternating the reservoirs using the turncock, the IOP could be acutely changed, keeping the water surface placid in the bottles. The other needle was connected to a pressure transducer for continuous monitoring of the actual IOP. Ten minutes after IOP was adjusted at 20 mm Hg, ONH blood flow was obtained by the hydrogen gas clearance method, and NBONH was immediately measured in the same eye after confirming no change in the electrode’s positioning and no visible bleeding. Thereafter, IOP was retained at 20 mm Hg (n = 4) or was changed to 40, 50, or 60 mm Hg (n = 4, each). After a 10-minute interval, the hydrogen gas clearance method and NBONH measurement were repeated.

**Time Course of NB Change for 60 Minutes and 25 Seconds after Acute Increase in IOP**

IOP was adjusted at 20 mm Hg and NBONH was serially monitored at 1-minute intervals for 10 minutes. Subsequently, the IOP was changed from 20 mm Hg to 40, 50, or 60 mm Hg (n = 6, each). NBONH was serially monitored at 1-minute intervals for the first 15 minutes and then at 30 and 60 minutes after the change in IOP. For comparison, the time course of changes in NB in the posterior choroid (NBchor) was also studied (n = 4). After IOP was adjusted to 20 mm Hg and held for 10 minutes, the IOP was manometrically increased to 50 mm Hg by changing the reservoirs. NBchor was serially monitored at 5- or 15-minute intervals for 60 minutes after the change in IOP. NBchor was measured at the posterior choroid one pupillary diameter below the ONH with the same-sized measurement fields as for the NBONH.

In a separate group of rabbits, the changes in NBav in the ONH for approximately 25 seconds after the acute increase in IOP from 20 to 50 or 60 mm Hg was studied with much higher time resolution. IOP was adjusted at 20 mm Hg for at least 10 minutes to confirm that stable NBONH results were obtained. Then, continuous recording of NBav at 0.125-second intervals was started. Approximately 5 seconds after the start of serial NBav measurement, the IOP was increased to 50 or 60 mm Hg by changing the reservoirs, and NBav recording was continued for 25 seconds (n = 10 or 6, respectively). Changes in NBav in the choroid for 25 seconds after the increase in IOP from 20 to 50 mm Hg was measured in the same manner (n = 6).

**Effects of a Calcium Antagonist**

Nilvadipine (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan), a dihydropyridine calcium antagonist, was continuously administered at the rate of 1 μg/kg per hour through the auricle vein of rabbits similarly prepared.

Before nilvadipine administration was started, IOP was adjusted at 20 mm Hg and NBONH and systemic parameters were determined as described earlier. Twenty minutes after nilvadipine administration was started, because blood pressure was reduced by the nilvadipine, IOP was readjusted to keep OPP unchanged from its level before the administration of nilvadipine in each rabbit (IOP1). After confirming that NBONH was stable, IOP was increased from IOP1 to the second level (IOP2), which was 30 or 40 mm Hg higher than IOP1. NBONH was measured just before and at 30 and 60 seconds, 1, 5, 10, 20, 30, 40, 50, and 60 minutes after the increase in IOP (n = 6). As a control, the same protocol was performed with the same volume of the vehicle solution instead of nilvadipine in a separate group of rabbits (n = 6).

Using other rabbits, changes in NBav for 25 seconds after the change in IOP from IOP1 to IOP2 were measured with high-time-resolution analysis according to the protocol described earlier, during the continuous administration of 1 μg/kg per hour nilvadipine (n = 6) or the vehicle solution (n = 6, control).

In these experiments, one investigator measured NBONH and NBav and another monitored IOP and systemic blood pressure. Both of them were masked to the treatment with nilvadipine or vehicle in each rabbit, and each was masked to the results obtained by the other.

**Effects of an NOS Inhibitor or Indomethacin**

After IOP was adjusted at 20 mm Hg and NBONH recorded as described for at least 10 minutes, serial recording of NBav, at 0.125-second interval was started. Approximately 5 seconds after the NBav recording was started, IOP was increased to 50 mm Hg and NBav recording was continued for the next 25 seconds. When the NBav recording finished, the IOP was returned to 20 mm Hg again. Subsequently, 30 mg/kg N-nitro-L-arginine (L-NAME), a nonselective NOS inhibitor, was intravenously injected from the auricular vein (n = 10). Thirty minutes after administration, the same protocol of change in IOP and serial NBav measurements was repeated. Using the same experimental design, the
Effects of intravenous administration of 5 mg/kg indomethacin was also studied \((n = 9)\). As a control, the same experiment was performed in a separate group of rabbits \((n = 10)\), to which a similar amount of physiological saline was administered instead of L-NAME or indomethacin. The investigator who measured NBav was masked to whether the rabbit had received L-NAME, indomethacin, or physiological saline. Because preliminary experiments revealed that the drugs given caused little effect on FABPm in Japanese albino rabbits, the second level of IOP was set at 50 mm Hg.

To compare the time course of changes in NBav before and after L-NAME or indomethacin administration, a time-parameter analysis was applied to the time course obtained in each rabbit. For each of the NBav curves, two time parameters, such as descending time \((T_d)\) and recovery time \((T_r)\), were defined as follows (Fig. 1). Serial NBav results obtained for 3 seconds just before the change in IOP from 20 to 50 mm Hg were averaged to obtain the baseline NBav. The difference between the baseline NBav and the minimum NBav was defined as NBreduction. If the minimum NBav was recorded at two or more different time points, the first time point was adopted. The time to obtain the minimum NBav from the change in IOP was defined as descending time. The time to recover the 90% of NBreduction from the minimum NBav was defined as recovery time. If the NBav showing the 90% of NBreduction was recorded at two or more different time points, the first time point was adopted.

**Effects of Sympathetic Nerve Amputation**

After systemic preparation as described earlier, except pupillary dilation in six rabbits, the vago sympathetic nerve trunk was bilaterally exposed and confirmed with ipsilateral mydriasis induced with electric stimulation of the trunk. Thereafter, a randomly chosen side of the nerve trunk was sectioned with surgical scissors, while the contralateral nerve remained. Approximately 1 hour after these preparations, the pupil was dilated with tropicamide, and the anterior chamber was cannulated for controlling and monitoring IOP as described earlier in one randomly chosen eye of each rabbit. After IOP was kept at 20 mm Hg and NBONH measured for at least 10 minutes, serial recording of NBav at 0.125-second intervals was started. Approximately 5 seconds after the NBav recording was started, the IOP was increased to 50 mm Hg and recording of NBav was continued for the next 25 seconds. Subsequently, the same protocol was performed in the contralateral eye after confirming no significant changes in FABPm. The time parameter analysis of the time course changes in NBONH was performed in the same manner as described earlier.

**RESULTS**

**Correlation between NBONH and ONH Blood Flow Measured by the Hydrogen Gas Clearance Method**

Because NBONH is a relative value, the change in NBONH due to the increase in IOP was compared to the change in blood flow rate obtained by the hydrogen gas clearance method in each rabbit. There was a significant correlation between the change in NBONH and the change in blood flow rate (Spearman’s rank correlation coefficient, \(R_s = 0.83\), \(P < 0.001\)). There were neither abnormal values nor significant changes in the systemic condition parameters. In a section of the ONH in which the hydrogen needle was inserted, neither major hemorrhage nor apparent destruction of the tissue structure was found.

**Time Course of NBONH Change before and after Acute Increase in IOP**

Figure 3 shows the time course of NBONH and OPP for 60 minutes before and after the change in IOP in rabbits. Increasing IOP showed little effect on NBONH 1 minute after IOP was increased in the groups in which the IOP was elevated to 40 or 50 mm Hg, whereas NBONH was significantly decreased and showed no recovery to the initial value in the group in which the IOP was elevated to 60 mm Hg (Wilcoxon signed rank test, \(P < 0.05\)). There were neither abnormal levels nor significant changes in the systemic parameters during the experiment. Figure 4 shows the time course changes in NBav and OPP for 60 minutes after change in IOP in rabbits. After the change in IOP, NBav and OPP decreased by approximately 25% and 50%, respectively.

Figure 5 shows an example of the recordings for 25 seconds of NBav and actual IOP before and after a change in IOP. NBav showed a transient decline and a rapid recovery to the initial level within several seconds. The mean change in NBav in the ONH after a change in IOP from 20 mm Hg to 50 or 60 mm Hg is shown in Figure 6, in which the data are plotted at intervals of 0.625-second. NBav showed a transient decrease during the first 3 seconds after the change in IOP when IOP was increased to 50 mm Hg, whereas no apparent recovery, except a slight one immediately after the change in IOP, was found when IOP was increased to 60 mm Hg. The mean change in NBav in the choroid after a change in IOP from 20 to 50 mm Hg is shown in Figure 7. Although a slight recovery was observed immediately after the change in IOP, no persistent recovery was found.
Effects of a Calcium Antagonist

In the experiment in which there was a 60-minute observation after the increase in IOP, baseline NBONH, which was obtained just before the change in IOP, was 10.1 ± 0.9 in the nilvadipine-treated rabbits, which was significantly higher than that in the vehicle-treated rabbits (8.7 ± 0.4; Mann-Whitney test, P = 0.045). In the nilvadipine-treated rabbits, NBONH showed an acute reduction of approximately 20% and %NBONH (the relative NBONH versus baseline) was significantly smaller than that in the vehicle-treated rabbits at each time point after the change in IOP (Mann-Whitney test with Bonferroni correction; P = 0.016, Fig. 8). Although FABP_m in the nilvadipine-treated rabbits was lower than that in the vehicle-treated rabbits at each time point, there was no significant difference in OPP, because IOP was set approximately 10 mm Hg lower in the nilvadipine-treated rabbits than in the vehicle-treated rabbits (Fig. 8). Other systemic parameters were not significantly different between the two groups (Table 1).

In the experiment with the 25-second observation after the increase in IOP, baseline NBav, which was obtained just before the change in IOP, was 10.1 ± 0.9 in the nilvadipine-treated rabbits, which was significantly higher than that in the vehicle-treated rabbits (8.7 ± 0.3, Mann-Whitney test, P = 0.045). In the nilvadipine-treated rabbits, NBav showed an acute reduction of approximately 20% and %NBav (the relative NBav versus baseline) was significantly smaller than that in the vehicle-treated rabbits at each time point after the change in IOP (Mann-Whitney test with Bonferroni correction: P = 0.016, Fig. 8). Although FABP_m in the nilvadipine-treated rabbits was lower than that in the vehicle-treated rabbits at each time point, there was no significant difference in OPP, because IOP was set approximately 10 mm Hg lower in the nilvadipine-treated rabbits than in the vehicle-treated rabbits (Fig. 8). Other systemic parameters were not significantly different between the two groups (Table 1).

In the experiment with the 25-second observation after the increase in IOP, baseline NBav, that was obtained just before the change in IOP was significantly higher in the nilvadipine-treated rabbits (10.1 ± 0.8) than in the vehicle-treated rabbits (9.0 ± 0.5; Mann-Whitney test, P = 0.014). NBav showed quick recovery after the change from IOP_1 to IOP_2 in the vehicle-treated rabbits, whereas the recovery was apparently restricted in the nilvadipine-treated rabbits (Fig. 9). Systemic parameters were almost similar to those in the experiment with a 60-minute observation period.

Effects of an NOS Inhibitor and Indomethacin

After treatment with l-NAME, the baseline NBav slightly but significantly decreased from 8.0 ± 0.3 to 7.4 ± 0.5 (Wilcoxon signed rank test, P = 0.028). The descending time did not significantly change after the administration of l-NAME (P > 0.1), whereas the recovery time was significantly prolonged compared with the pretreatment level (P = 0.012) and the increase was significantly greater than that in saline-treated rabbits (Mann-Whitney test, P = 0.023; Table 2). On the other hand, in the indomethacin and saline groups, neither baseline NBav, descending time, nor recovery time after the treatments was significantly different from that before the treatments (P > 0.1). FABP_m and other systemic parameters did not significantly change before and after the treatment with l-NAME, indomethacin, or physiological saline (Table 3).

FIGURE 3. Time course of changes in the relative NBONH (A) compared with the baseline value (% NBONH) and OPP (B) for 60 minutes after change in IOP from 20 mm Hg to 40, 50, or 60 mm Hg in rabbits. Data are expressed as the average ± SE (n = 6, each). Increasing IOP showed little effect on NBONH in the groups in which IOP_2 was 40 or 50 mm Hg, whereas NBONH was significantly decreased compared with the initial value in rabbits in which IOP was increased to 60 mm Hg (Wilcoxon signed rank test, P < 0.05).

FIGURE 4. Time course of changes in the relative NBav (A) compared with baseline (% NBav) and OPP (B) for 60 minutes after change in IOP from 20 to 50 mm Hg. Data are expressed as the average ± SE (n = 4).

FIGURE 5. An example of the recordings for 25 seconds of NBav and IOP before and after change in IOP from 20 to 50 mm Hg (arrow).
Effects of Sympathetic Nerve Amputation

The baseline NB_{av} at IOP of 20 mm Hg in eyes in which the sympathetic nerve was amputated was significantly larger than that in the contralateral untreated eyes (9.6 ± 1.8 vs. 8.3 ± 1.0; Wilcoxon signed rank test, P = 0.027, Table 4). However, there was no significant difference in descending or recovery times in the time course changes of NB_{av} between the eyes with sympathetic nerve amputation and the contralateral control eyes. Systemic parameters including FABP_{m} did not significantly change during the experiment (Table 4).

**DISCUSSION**

The NB index obtained by laser speckle tissue circulation analysis is primarily a quantitative index of tissue blood velocity.
However, it has also been found to correlate with the blood flow rate in the iris, retina, and choroid. The rabbit NBONH had good correlation with the results obtained with the hydrogen gas clearance method, in which a needle electrode was inserted into the ONH to the depth of approximately 0.7 mm, regarding the changes after systemic administration of endothelin-1, nilvadipine, or inhalation of CO₂ in the present study, the change in NBONH and that in ONH blood flow measured by the hydrogen gas clearance method also correlated significantly (R² = 0.85, P < 0.001, Fig. 2). The blood flow rate in the rabbit ONH at the IOP of 20 mm Hg was estimated to be 115 mL/min per 100 g by the hydrogen gas clearance method in which the electrode was inserted to a depth of 0.7 mm in the ONH tissue. This finding suggests that the present NBONH data are likely to reflect blood flow changes in the rabbit ONH, not only from the superficial layers but also in layers beneath the lamina scleralis.

In the present study, the time course of changes in ONH circulation was documented. NBONH quickly decreased and immediately recovered within several seconds after an acute increase in IOP from 20 mm Hg to 40 or 50 mm Hg (Figs. 3 and 6). These findings were consistent with the previous works in which the ONH blood flow recovered within 1 minute after the change in OPP in cats. Moreover, very short-term changes just after increase in IOP were also found in the present study. These results obtained in the ONH contrasted with those in the posterior choroid (Fig. 4), in which NBOCHO was decreased and recovered within 1 minute after the increase in IOP.

### Table 2. Baseline NBONH, Descending Time, and Recovery Time before and after the Administration of l-NAME, Indomethacin, or Physiological Saline

<table>
<thead>
<tr>
<th>l-NAME</th>
<th>Indomethacin</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rabbits</td>
<td>10, 10</td>
<td>10</td>
</tr>
<tr>
<td>Baseline-NBONH (mm Hg)</td>
<td>8.0 ± 0.3, 7.9 ± 0.5, 8.0 ± 0.4</td>
<td>7.4 ± 0.3, 7.5 ± 0.5, 8.2 ± 0.5</td>
</tr>
<tr>
<td>Post</td>
<td>8.0 ± 0.3, 7.9 ± 0.5, 8.0 ± 0.4</td>
<td>7.4 ± 0.3, 7.5 ± 0.5, 8.2 ± 0.5</td>
</tr>
<tr>
<td>Change</td>
<td>-0.5 ± 0.3, -0.3 ± 0.6, 0.2 ± 0.5</td>
<td>0.1 ± 0.3, 0.2 ± 0.6, 0.7 ± 0.5</td>
</tr>
<tr>
<td>Descending Time (sec)</td>
<td>1.69 ± 0.19, 1.88 ± 0.17, 1.75 ± 0.40</td>
<td>2.03 ± 0.16, 2.44 ± 0.19</td>
</tr>
<tr>
<td>Post</td>
<td>1.69 ± 0.19, 1.88 ± 0.17, 1.75 ± 0.40</td>
<td>2.03 ± 0.16, 2.44 ± 0.19</td>
</tr>
<tr>
<td>Change</td>
<td>0.25 ± 0.25, 0.14 ± 0.17, -0.31 ± 0.50</td>
<td>0.19 ± 0.20, 0.33 ± 0.40</td>
</tr>
<tr>
<td>Recovery Time (sec)</td>
<td>2.56 ± 0.35, 2.18 ± 0.31, 2.00 ± 0.20</td>
<td>2.27 ± 0.20, 2.38 ± 0.24</td>
</tr>
<tr>
<td>Post</td>
<td>2.56 ± 0.35, 2.18 ± 0.31, 2.00 ± 0.20</td>
<td>2.27 ± 0.20, 2.38 ± 0.24</td>
</tr>
<tr>
<td>Change</td>
<td>1.13 ± 0.35, 0.97 ± 0.22, 0.38 ± 0.28</td>
<td>0.94 ± 0.23, 1.01 ± 0.36</td>
</tr>
</tbody>
</table>

Data are the mean ± SE. Pre or post are the data obtained before or 30 minutes after the administration of l-NAME, indomethacin, or physiological saline, respectively. Change is the difference between readings obtained before and after the administration.

### Table 3. Systemic Parameters before and after the Administration of l-NAME, Indomethacin, or Saline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>l-NAME</th>
<th>Indomethacin</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>FABPONH (mm Hg)</td>
<td>92.2 ± 2.8, 96.4 ± 2.2, 95.6 ± 3.4</td>
<td>96.3 ± 5.2, 101.5 ± 4.9, 102.3 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>96.3 ± 5.2, 101.5 ± 4.9, 102.3 ± 3.2</td>
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<td></td>
</tr>
<tr>
<td>Pulse Rate (min)</td>
<td>247 ± 8, 250 ± 6, 259 ± 8</td>
<td>250 ± 10, 257 ± 8, 240 ± 8</td>
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<tr>
<td>Pre</td>
<td>247 ± 8, 250 ± 6, 259 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>250 ± 10, 257 ± 8, 240 ± 8</td>
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<tr>
<td>pH</td>
<td>7.38 ± 0.01, 7.41 ± 0.02, 7.39 ± 0.01</td>
<td>7.38 ± 0.01, 7.38 ± 0.02, 7.44 ± 0.02</td>
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<tr>
<td>Pre</td>
<td>7.38 ± 0.01, 7.41 ± 0.02, 7.39 ± 0.01</td>
<td></td>
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</tr>
<tr>
<td>Post</td>
<td>7.38 ± 0.01, 7.38 ± 0.02, 7.44 ± 0.02</td>
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<td></td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>36.2 ± 1.1, 35.1 ± 1.4, 35.4 ± 1.2</td>
<td>35.2 ± 1.2, 37.1 ± 1.1, 34.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>36.2 ± 1.1, 35.1 ± 1.4, 35.4 ± 1.2</td>
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<td></td>
</tr>
<tr>
<td>Post</td>
<td>35.2 ± 1.2, 37.1 ± 1.1, 34.0 ± 1.5</td>
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<tr>
<td>Body temperature (°C)</td>
<td>38.1 ± 0.1, 38.0 ± 0.2, 38.1 ± 0.1</td>
<td>37.4 ± 0.2, 38.0 ± 0.1, 38.1 ± 0.2</td>
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</tr>
<tr>
<td>Pre</td>
<td>38.1 ± 0.1, 38.0 ± 0.2, 38.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>37.4 ± 0.2, 38.0 ± 0.1, 38.1 ± 0.2</td>
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</tr>
</tbody>
</table>

Data are the mean ± SE.

Although it should be difficult to accurately decide how deep the laser penetrates into the rabbit ONH tissue, Koelle et al. reported that infrared laser (wavelength: 811 nm; power: 2 mW) penetrated to a depth of approximately 1 mm in the cat optic nerve. On the contrary, Petrig et al. reported that laser Doppler flowmetry (wavelength approximately 800 nm) is predominantly sensitive to blood flow changes in the superficial layers of the monkey ONH. In the present study, NBONH significantly correlated with the results obtained by the hydrogen gas clearance method in which the electrode was inserted to a depth of 0.7 mm in the ONH tissue. This finding suggests that the present NBONH data are likely to reflect blood flow changes in the rabbit ONH, not only from the superficial layers but also in layers beneath the lamina scleralis.

### Table 4. Effects of Severing the Sympathetic Nerve on Baseline NBONH, the Descending and Recovery Times, and Systemic Conditions

<table>
<thead>
<tr>
<th>Sympathetic Nerve Severed</th>
<th>(+)</th>
<th>(−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline-NBONH (mm Hg)</td>
<td>9.6 ± 1.8</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>Descending Time (sec)</td>
<td>1.88 ± 0.51</td>
<td>1.72 ± 0.50</td>
</tr>
<tr>
<td>Recovery Time (sec)</td>
<td>2.03 ± 0.29</td>
<td>2.19 ± 0.18</td>
</tr>
<tr>
<td>FABPONH (mm Hg)</td>
<td>92.5 ± 2.1</td>
<td>92.6 ± 2.5</td>
</tr>
<tr>
<td>Post</td>
<td>92.5 ± 2.1, 92.6 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Pulse Rate (/min)</td>
<td>272 ± 6</td>
<td>275 ± 7</td>
</tr>
<tr>
<td>Pre</td>
<td>272 ± 6</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>275 ± 7</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.01</td>
<td>7.42 ± 0.01</td>
</tr>
<tr>
<td>Pre</td>
<td>7.43 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>7.42 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>38.7 ± 1.2</td>
<td>39.4 ± 0.9</td>
</tr>
<tr>
<td>Pre</td>
<td>38.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>39.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>35.0 ± 0.1</td>
<td>35.0 ± 0.1</td>
</tr>
<tr>
<td>Pre</td>
<td>35.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>35.0 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are the mean ± SE error (n = 6).

* Significantly different from pretreatment levels, at P = 0.0284 and 0.0117, respectively (Wilcoxon signed rank test).
† Significantly greater than the level in saline-treated rabbits (P = 0.0232, Mann-Whitney test).

### Figure 9. Time course of changes in the relative NBONH compared with baseline (%NBONH) obtained for 25 seconds after change in IOP from IOP1 to IOP2, in nilvadipine-treated rabbits and vehicle-treated rabbits. Data are expressed as the average ± SE (n = 6, each).
showed little recovery after the increase in IOP from 20 to 50 mm Hg (OPP decrease from 60 to 30 mm Hg). However, NB\textsubscript{ONH} decreased by approximately 25% under the condition that OPP decreased by approximately 50%, and NB\textsubscript{a} in the choroid showed a slight recovery response immediately after the change in IOP in the 25-second experiment (Fig. 7). These findings suggest that the choroidal circulation may not be completely passive against changes in OPP, although its autoregulatory mechanism was apparently weaker than that in the ONH. In contrast, when IOP was increased to 60 mm Hg (OPP decreased to 20 mm Hg), no recovery was seen in NB\textsubscript{ONH} (Fig. 3). Decrease in NB\textsubscript{ONH} was considerably smaller, however, than that in OPP (35% vs. 66%). Although change in NB\textsubscript{ONH} tended to underestimate the IOP-induced reduction in the ONH blood flow (Fig. 2), this finding may suggest that some autoregulatory mechanism still has effects. The current results were consistent with the previously reported ranges of OPP in which autoregulation of the rabbit ONH was observed.

In the present study, NB\textsubscript{ONH} in the nilvadipine-treated rabbits was significantly higher than that in the vehicle-treated rabbits, suggesting an increase in ONH blood flow velocity induced by nilvadipine treatment. However, response against the acute decrease of perfusion pressure (i.e., acute increase in IOP) was apparently impaired. Nilvadipine is a Ca\textsuperscript{2+} antagonist classified in the dihydropyridine group, blocks L-type calcium channels, and is relatively selective of cerebral arteries. Calcium antagonists impair influx of Ca\textsuperscript{2+} into the vascular smooth muscles and usually increases peripheral circulation. Recent studies using isolated vessels including rabbit cerebral arteries showed that many kinds of Ca\textsuperscript{2+} antagonists abolish or attenuate the stretch-induced contraction of vascular smooth muscles. An in vivo study revealed that a Ca\textsuperscript{2+} antagonist (nimodipine) inhibits autoregulation of cerebral blood flow against arterial pressure increase by 40 mm Hg in cats and monkeys. To our knowledge, however, no studies have investigated the effects of Ca\textsuperscript{2+} antagonists on the time course of the change in ONH circulation after an acute increase in IOP (and decrease in OPP).

The current results suggest that the Ca\textsuperscript{2+} antagonist reduces the basal tone of the vascular smooth muscle, as documented by an increase in the baseline NB\textsubscript{ONH} and attenuation of the additive relaxation necessary for the quick recovery response of a decrease in OPP. To maintain a stable vasodilating effect of nilvadipine, the drug was continuously administered at the rate of 1 μg/kg per hour in the present study. Because nilvadipine is a lipophilic agent and is easily and strongly bound to the receptors on the cell membrane, its effect on the peripheral vessels does not directly follow its concentration in the blood. In animal experiments, the optimum concentration of a bolus intravenous nilvadipine for reducing systemic arterial pressure ranged between 0.1 and 10 μg/kg, and the effect continued for at least 1 hour. Thus, the continuous administration of 1 μg/kg nilvadipine per hour was adopted for the current experiments. Although direct comparison between bolus or continuous intravenous and oral administration is usually difficult, the maximum blood concentration after oral administration of a 4-mg tablet of nilvadipine in normal humans is 3.5 ng/ml, which roughly corresponds to that after a bolus administration of nilvadipine at 0.5 μg/mL per kilogram in rabbits. Because 2 or 4 mg oral nilvadipine is the clinical dose for the treatment of systemic hypertension, the current dosage in rabbits should roughly correspond to the ordinary clinical condition.

In the nilvadipine-treated rabbits, NB\textsubscript{ONH} decreased by approximately 20% after an increase in IOP from 20 to 50 mm Hg, corresponding to an OPP decrease from 65 to 32 mm Hg (approximately 50% decrease). The apparent dissociation between a 20% decrease in NB\textsubscript{ONH} and a 50% decrease in OPP suggests that the vascular system in the ONH tissue is not completely passive against the change in OPP, even after nilvadipine treatment at the present dose, and that other factors also may be involved in the maintenance of constant ONH circulation against an acute decrease in OPP. Because many kinds of Ca\textsuperscript{2+} antagonists are commonly used for the treatment of cardiovascular or cerebral diseases, the possibility should be noted that response to OPP changes may be somewhat modified in patients taking those drugs. For example, the acute increase in IOP due to an episode of acute angle-closure glaucoma or secondary glaucoma may exert more unfavorable influences in patients who are taking systemic Ca\textsuperscript{2+} antagonists.

NO and prostacyclin are released from the vascular endothelium according to the changes in the shear stress and play vital roles in local control of the vascular tone. Because complete inhibition of the endothelium function cannot be obtained in living animals, we tested the effects of L-NAME (a nonselective inhibitor of NO synthesis) and indomethacin (an inhibitor of synthesis of prostaglandins including prostacyclin) on the quick recovery response in the ONH circulation after the changes in OPP. L-NAME showed a slight but significant retarding effect on the quick recovery of the ONH circulation, whereas indomethacin showed no effect. The doses of L-NAME and indomethacin used in this study were equivalent or larger than those used in previous studies in which their vasoactive effects were certified in rabbits. In the present study, baseline NB\textsubscript{ONH} showed a slight, but significant reduction after administration of L-NAME, suggesting that NO synthesis was at least partly inhibited. However, no manifest change in the blood pressure may suggest only a partial inhibition of NO synthesis. The reduction in NB\textsubscript{ONH} and the change in blood pressure after administration of L-NAME in the present study were apparently smaller than those obtained in conscious albino rabbits. The anesthesia used in the present study may have some influence on the vascular basal tone or vasoactive reaction to L-NAME.

Giddday et al. reported that an NOS inhibitor (N\textsuperscript{6}-monomethyl-L-arginine) showed no significant influences on the autoregulatory vasodilatation of the newborn pig retinal artery caused by systemic hypoxia, hypotension, or hypercapnia. In contrast, Buerk et al. found that NO is important for functional hyperemia (vasodilatation) of the cat ONH circulation during increased neuronal activity with flickering light stimuli to the eye, but Buerk and Riva found that it is not essential for vasomotion in unstressed conditions. Because the effect of the NOS inhibitor on the rapid recovery of ONH circulation after an acute increase in IOP (decrease in OPP) was found to be small under the current experimental conditions, it is suggested that NO is not a main mediator for the reaction, at least in this species of animal. However, there remains a possibility that Japanese albino rabbits are not a suitable animal species in which to study the role of NO in ONH circulation.

In the current experiment involving the amputation of the sympathetic nerve, the alteration of IOP and measurements of NB\textsubscript{ONH} were performed approximately 1 hour after the nerve was severed. Therefore, it is supposed that neither the effect of the ganglionectomy, which manifests as a degenerating release of norepinephrine from adrenergic nerve fiber terminals and usually occurs at least 12 hours after ganglionectomy, nor the denervation supersensitivity that occurs approximately 10 days after injury occurred during the experimental period. It has been concluded in earlier reports that sympathetic nerves in mammalians innervate the central retinal artery up to the ONH, but not beyond, whereas all uveal vascular beds are...
innervated. However, recent study using electron microscopy and fluorescent examination has revealed that sympathetic nerve innervation is found in the retinal arteries beyond the lamina cribrosa in rabbit eyes. Similar to the results of experiments using the microsphere technique, sympathetic stimulation reduces the uveal blood flow, but does not affect the blood flow in the retina and optic nerve of cats, and monkeys. Other investigators have reported that the cat retinal blood flow increases after sympathectomy. Using the microsphere technique, Linder showed that autoregulation against systemic hypotension is partly impaired by sympathetic stimulation in the rabbit retina, but not in the optic nerve. The results of the present study obtained using the laser speckle method suggest that cutting the cervical sympathetic chain has a small but significant accelerating effect on the basal level of ONH circulation, whereas it shows no significant effects on its quick recovery response after acute changes in IOP.

We performed several experiments in the present study, and some of the systemic parameters such as PO2 during the sympathetic nerve amputation experiment were different from those in other experiments (Tables 1, 3, and 4). Although we do not have a good explanation for this difference, a main reason would be the surgical procedures used in this experiment for amputation of the sympathetic nerve, and the time intervals between the induction of general anesthesia and the NB experiments differed between this experiment and others because of this surgery. None of the systemic parameters, however, exceeded the normal ranges of healthy rabbits.

In conclusion, the present series of experiments using the laser speckle method indicate that recovery in the ONH circulation is accomplished in the first several seconds after an acute increase in IOP, and that the influx of Ca2+–related vascular smooth muscle relaxation was confirmed to play a role in the response. The production of NO or prostaglandins, or sympathetic nervous system appeared to have slight effects on it. The laser speckle method was found to be suited to noninvasive monitoring of the changes in ONH circulation with high time resolution and its process after various stimulations and to investigate factors relating to them. There are differences regarding anatomy and blood supply in the ONH between rabbits and primates or humans, despite similarities in the arterial supply. Therefore, the current results may not be directly applied to the ONH circulation of primates or humans, but should provide useful information for future laboratory studies and probably for clinical settings.

References


