Optic Cup Enlargement Followed by Reduced Optic Nerve Head Circulation after Optic Nerve Stimulation

Tetsuya Sugiyama,1 Hideaki Hara,2 Hidehiro Oku,1 Shunji Nakatsuji,3 Takashi Okuno,1 Masaaki Sasaoka,2 Takashi Ota,2 and Tsunehiko Ikeda1

PURPOSE. To investigate changes in optic nerve head (ONH) circulation, visual evoked potentials (VEPs), and ONH cupping after stimulation of the optic nerve.

METHODS. Electrodes were fixed above the optic chiasma in rabbits under general anesthesia. Screw-type electrodes for VEP recording were fixed on the dura. ONH circulation, intraocular pressure (IOP), and blood pressure (BP) were measured after the passage of a current of 0.1 mA for 0.1 second (weak stimulation), 1 mA for 1 second (moderate), 5 mA for 10 seconds (strong), or 25 mA for 10 seconds (severe). Normalized blur (NB), indicative of tissue blood flow and velocity, was measured in the ONH after each stimulation, by using a laser speckle circulation analyzer. Changes in VEP and ocular fundus were also recorded. The ratio of cup area (CA) to disc area (DA) was measured before and 4 weeks after stimulation. After all experiments, the ONH was histologically examined.

RESULTS. Weak stimulation increased NB in ONH for 10 minutes, whereas strong or severe stimulation significantly decreased NB for a longer time, in a dose-dependent manner. BP showed no significant change, except with severe stimulation. IOP was not significantly changed. VEP amplitude was reduced 30 minutes after strong stimulation. The CA-to-DA ratio was significantly increased 4 weeks after strong stimulation. In some rabbits, disc hemorrhage occurred, followed by enlargement of disc cupping, with slight gliosis.

CONCLUSIONS. Electrical stimulation of the optic nerve changed ONH circulation and VEPs and increased disc cupping. This technique warrants further investigation as an experimental model for normal-tension glaucoma. (Invest Ophthalmol Vis Sci. 2001;42:2843–2848)

Normal-tension glaucoma (NTG) is a common type of glaucoma, especially in Japan, where it is prevalent.1 Although an animal model of high-pressure glaucoma has already been established,2,3 an experimental model of NTG has not. In NTG, disturbance in optic nerve head (ONH) circulation is suspected to be one of the causal factors.4,5 It has also been reported that patients with NTG, especially those with focal ischemia, often have migraine or vasospastic syndrome.6–9 Conversely, visual field loss often occurs in patients with migraine.10,11 These phenomena suggest a relationship between NTG and migraine, which may have a similar pathogenesis.

On the other hand, cortical spreading depression (CSD), an experimental model of migraine, can be induced by electrical stimulation of the cortex, which is followed by changes in cortical blood flow.12–14 In this study, changes in ONH circulation, VEPs, and ONH cupping were investigated after electrical stimulation of the optic nerve in rabbits to advance the establishment of an experimental model for NTG.

MATERIALS AND METHODS

Animals

Male albino rabbits weighing 2.5 to 3.0 kg were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). They were housed in an air-conditioned room (22 ± 1°C with 66% ± 5% humidity), with a 12-hour light-dark diurnal cycle and access to food and water ad libitum. They were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgery

With the rabbit under general anesthesia with intravenous pentobarbital sodium (Nembutal; Abbott Laboratories, Chicago, IL), the head was fixed in a stereotaxic apparatus (Summit Medical, Tokyo, Japan). Parts of the skull were ground off with an electric drill (Mini Gold; Natume, Tokyo, Japan) and a bipolar stimulating electrode (Neuroscience, Osaka, Japan) was buried in the brain with its tip projecting almost into the optic chiasma, according to a stereotaxic atlas of the rabbit brain15 (Fig. 1). Electrodes for VEP recording were placed on the dura over the cortical visual area. All electrodes were fixed to the skull with dental cement (Unifast 2; GC Corp., Tokyo, Japan).

Electrical Stimulation

At least a week after surgery, conscious rabbits were placed in holding boxes and their brains electrically stimulated through the implanted bipolar electrodes. The stimulation conditions were ranked in four grades: direct current of 0.1 mA for 0.1 second (weak stimulation), 1 mA for 1 second (moderate), 5 mA for 10 seconds (strong), and 25 mA for 10 seconds (severe). Each rabbit was stimulated under two or three conditions. Rabbits that were not stimulated at all served as the control.

Measurement of ONH Tissue Blood Flow, Intraocular Pressure, and Blood Pressure

The tissue blood flow of the ONH was measured with a laser speckle tissue circulation analyser. The details of this apparatus have been reported by Tamaki et al.16 and Fuji.17 Briefly, scattered laser light was projected onto an image sensor, where a laser speckle pattern appeared. The normalized blur (NB) obtained with this apparatus was...
equivalent to a quantitative index of the blurring of the speckle pattern and was an indicator of tissue blood velocity. The relative change of the NB showed a strong correlation with the change in the ONH tissue blood flow measured by the hydrogen gas clearance method, suggesting that change in NB is indicative of change in blood flow. Rabbits were placed in holding boxes, and the measurements described in the following section were obtained with the animals under local anesthesia with a drop of 0.4% oxybuprocaine hydrochloride (Benoxil; Santen Pharmaceutical, Osaka, Japan).

For measurement of ONH tissue blood flow, the NB over a 0.72 x 0.72-mm area of the ONH free of surface vessels was averaged in a randomly selected eye after mydriasis with a drop of 0.4% tropicamide (Mydrin M; Santen Pharmaceutical). It took 0.18 seconds to record 98 scans to obtain one NB value. The NB at each time was calculated as the average of five successive measurements. NB was measured for 90 minutes after each stimulation. Intraocular pressure (IOP) was measured with a calibrated pneumatonometer (Alcon, Tokyo, Japan) in the contralateral eye to that used for blood flow measurement.

One of the auricular arteries was cannulated with a polyethylene tube (SP28; Natume, Tokyo, Japan), with the animal under local anesthesia with 2% lidocaine (Xylocaine spray; Fujisawa Pharmaceutical, Tokyo, Japan) for monitoring mean arterial BP, according to the following calculation: [diastolic BP + 1/3(systolic BP – diastolic BP)].

**VEP Recording and Analysis**

Approximately 1 week after the experiment just described, VEPs were recorded before and 30 minutes after each electrical stimulation except the severe one. Before each recording, dark adaptation was allowed for 30 minutes. The monitoring method has been described in detail. Briefly, VEPs were recorded from the active electrode on visual area 1 by the summation of 32 responses to a 0.6-J light stimulus at 1 Hz. The first negative peak with a latency of 20 msec was defined as N1, and its amplitude and latency were measured as indicators of visual function. Analogue data were recorded using a rectilinear plotter pen system and were simultaneously stored and digitized, using a microcomputer (MacLab 2e; Apple Computer, Cupertino, CA).

**Photographs of Ocular Fundus and Assessment of Morphologic Change of ONH**

Before and 1, 7, 14, and 28 days after each stimulation, photographs of the ocular fundus were taken with a fundus camera. The morphology of the ONH was assessed with fundus pictures taken with the ONH centered at 45° of visual angle from different angles before and 4 weeks after the strong stimulation. The method has been described in detail elsewhere. Briefly, the optic disc area (DA) and cup area (CA) were measured by calculating the number of pixels in each area on the computer display, and the CA-to-DA ratio was defined as an index of cup enlargement.

**Histologic Examination**

Approximately 1 month after the experiments, rabbits were killed with a lethal dose of pentobarbital sodium and their eyes and brains examined histologically. Brains of sham control rabbits that had not been stimulated were also examined.
The eye was enucleated and fixed in 0.1 M phosphate buffer (pH 7.4) containing 1% glutaraldehyde and 4% formaldehyde for 10 minutes and then cut at the ONH parallel to the medullary rays followed by fixation for 12 hours. After refixation with 10% neutral buffered formalin solution for 24 hours, the tissue was embedded in paraffin, cut in 4-μm sections, and stained with hematoxylin and eosin (H&E).

Statistics
Data are expressed as the mean ± SE. Statistical analysis of blood flow, IOP, and BP was performed using a two-way analysis of variance (ANOVA) for repeated measurements. If a statistically significant difference was detected, further assessment was made with a one-way ANOVA followed by Dunnett’s test. Student’s t-test was used for analysis of the VEP data and CA-to-DA ratio. A difference was considered significant at \( P < 0.05 \).

RESULTS
Changes in ONH Tissue Blood Flow, IOP, and BP
The changes in NB after each stimulation are shown in Figure 2. A two-way ANOVA for repeated measurements showed significant differences between the control and weak, control and strong, and control and severe stimulations. A one-way ANOVA followed by Dunnett’s test showed that weak stimulation significantly increased the NB at 5 and 10 minutes, whereas strong and severe stimulation decreased the NB for 20 to 60 minutes in a dose-dependent manner. Moderate stimulation had no significant effect on the NB. Each stimulation did not change IOP significantly (data not shown). The changes in BP after each stimulation are shown in Figure 3. A two-way ANOVA for repeated measurements showed significant differences only between the control and severe stimulation. In a one-way ANOVA followed by Dunnett’s test, severe stimulation was found to significantly increase BP for 45 minutes.

Changes in VEP
The changes in amplitude of VEPs after each stimulation are shown in Figure 4. Student’s paired t-test showed that the strong stimulation significantly reduced amplitude, but the control, weak, or middle levels did not change it. Latency of VEPs showed changes similar to amplitude, but there was no significance in its change.

Changes in Ocular Fundus
A typical short-term change in ocular fundus is shown in Figure 5. Blood vessels in the ONH and retina were obvi-

**Figure 2.** Changes of the NB in the ONH after electrical stimulation: (□) weak (0.1 mA for 0.1 second); (▲) moderate (1 mA for 1 second); (■) strong (5 mA for 10 seconds); (●) severe (25 mA for 10 seconds); (○) control (no stimulation). Data are expressed as the mean ± SE for five to eight rabbits. There were significant differences between the control and weak, control and strong, and control and severe stimulations (two-way ANOVA for repeated measurements). *Significant differences from the control group (\( P < 0.05 \); Dunnett’s test).

**Figure 3.** Changes in mean blood pressure (MBP) after electrical stimulation. Symbol key is the same as Figure 2. There was a significant difference between the control and severe stimulation (two-way ANOVA for repeated measurements). *Significant differences from the control group (\( P < 0.05 \); Dunnett’s test).

**Figure 4.** Changes in the amplitude of VEP after electrical stimulation. Symbol key is the same as Figure 2. There was a significant difference between amplitudes recorded before and after the strong stimulation (* \( P < 0.05 \); paired t-test). There were no significant differences between amplitudes before and after the weak or moderate stimulation.
ously constricted, and the ONH became pale after the severe stimulation. A typical long-term change in ocular fundus is shown in Figure 6. ONH hemorrhage was observed a day after the strong stimulation (Fig. 6A) and the optic cup was enlarged 4 weeks later (Fig. 6B). A similar ONH hemorrhage was detected in two of six eyes after strong stimulation. The CA-to-DA ratio was significantly increased 4 weeks after strong stimulation, and a significant difference was detected between the changes in the ratios of stimulated eyes and sham control eyes (Table 1).

**Histologic Findings**

Typical histologic change in the eye is shown in Figure 7. In some animals with a strongly stimulated eye, optic cup enlargement was observed with slight gliosis in the prelaminar region. Nevertheless, there was no significant necrosis in neurons and axons in the retrolaminar region of the optic nerve. No particular change was found in any layers, including the ganglion cell layer in the retina. Neither obstruction nor injury was seen in the vessels in the ONH. There was no change in the sham control eye (Fig. 8).

A histologic evaluation of the brain revealed that there were only necrotic lesions between right and left lateral ventricles, which seemed to be mechanically induced by insertion of the electrode for stimulation. But no obvious abnormality was found in the optic chiasma and the nearby optic nerve or in the visual area of the cerebral cortex.

**DISCUSSION**

An experimental NTG model has not yet been established, partly because the pathophysiology of NTG is currently not well known. Construction of an NTG model may lead not only to an understanding of NTG pathogenesis, but also to the development of new therapies. Vascular changes elicited by causes other than IOP, for example ocular vasoconstriction, are suspected to play an important role in the pathogenesis of NTG.21,22 It has been reported that systemic administration of calcium channel blockers with the ability to increase ocular blood flow could improve visual field defects in patients with NTG or prevent the development of such defects.23–25 Moreover, there are some reports that continuous reduction of ONH blood flow, induced by continuous or repeated administration of endothelin-1, could contribute to the enlargement of the ONH cupping seen in NTG.20,26 We speculate that electrical stimulation of the optic nerve could affect the ONH blood flow and induce functional and morphologic changes of the ONH.

In the present study, the effect of electrical stimulation of the optic nerve on tissue blood flow in the ONH of conscious rabbits was determined using the laser speckle method. This noninvasive method for two-dimensional measurement of tissue circulation in the ocular fundus has recently been developed in Japan.16,17 The NB in the ONH obtained by this method is indicative not only of tissue blood velocity but also...
of tissue blood flow. By this method, we probably cannot discern the tissue circulation of the superficial and deeper portions of the ONH, because only a weak correlation was reported between the blood flow indexes, as measured by laser speckle method and scanning laser Doppler flowmetry. The latter measures the circulation in the more superficial portion of the ONH, which is supplied by the branches originating from the central retinal artery. The laser speckle method measures not only the superficial but also deeper portion of the ONH, which is mainly supplied by the short posterior ciliary artery.

There have been no previous reports on the effect of electrical stimulation of the optic nerve on ONH circulation. However, there are some reports on the change in cerebral blood flow by electrical stimulation of lateral frontal cortex in an experimental model of migraine. Some investigators have reported that transient increase in cerebral blood flow is followed by a longer lasting decrease. Others have reported that transient hyperemia succeeds oligemia in a CSD model.

The present study showed that weak stimulation temporarily increased the ONH blood flow and in contrast, strong or severe stimulation decreased it for a longer time. In particular, severe stimulation induced a significant decrease at least until 60 minutes after stimulation. Thus, a more prolonged decrease might be induced, although this was not determined. In addition, the mechanism by which ONH blood flow was decreased is still unknown. The blood flow change in the whole eye and the total brain should be examined in the future. The changes in ONH circulation were probably caused mainly through neural pathways, not through general hemodynamics, because there were no particular changes in blood pressure except for the severe stimulation. It has been demonstrated that nitric oxide and excitatory amino acids, including glutamate, are related to changes in cortical blood flow in CSD. There are also some reports showing the relationship of glaucoma with elevated glutamate level in the vitreous body or increased nitric oxide synthase in the ONH. These substances may be involved in the mechanisms producing the results obtained in our study and should be further investigated. In any case, we have demonstrated for the first time that diminished ONH circulation can be elicited by stimulation of the optic nerve. This phenomenon supports that our model corresponds to the pathophysiology of NTG, because disturbed ONH blood flow is also observed in glaucoma and decreased ONH blood flow at night is related to the development of a visual field defect in NTG (Okuno et al; unpublished data, 2000).

We analyzed VEPs to evaluate the change in visual function after electrical stimulation of the optic nerve. VEP reflects electrical activity of the visual pathway from the retina through the optic nerve to the visual cortex. The condition of the central retinal region is selectively represented in the VEPs, as is ganglion function. The present study revealed that the amplitude of N1 in VEP was reduced by strong stimulation of the optic nerve. This result objectively showed that disturbed visual function may be caused, at least in part, by reduced ONH blood flow.

It has been reported that disc hemorrhage often occurs in NTG and also precedes retinal nerve fiber bundle and visual field defects. In our study, disc hemorrhage, which was followed by an enlarged optic cup with slight gliosis, was detected in some of the rabbits after electrical stimulation. In addition, ONH cupping was significantly increased after the stimulation without significant IOP change. Histologic evaluation suggested that change in the ONH was not induced by mechanical vessel obstruction but probably was produced by pathologic constriction. These results seem to be consistent with the characteristic changes observed in patients with NTG. That any significant changes in the retina, including in ganglion cell layer, were not found in the present study may be different from findings in NTG-affected eyes. Characteristic changes in the retina could be induced in the later period although this has not been ascertained yet.

In summary, we found that strong electrical stimulation of the optic nerve reduced ONH circulation, suppressed the amplitude in VEPs, and enlarged the optic cup without IOP elevation, sug-

**TABLE 1. CA-to-DA Ratio in Each Animal**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>% of Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.7</td>
<td>25.3</td>
<td>122.2</td>
</tr>
<tr>
<td>2</td>
<td>31.3</td>
<td>42.5</td>
<td>135.8</td>
</tr>
<tr>
<td>3</td>
<td>17.2</td>
<td>20.8</td>
<td>120.9</td>
</tr>
<tr>
<td>4</td>
<td>26.1</td>
<td>30.4</td>
<td>116.5</td>
</tr>
<tr>
<td>5</td>
<td>37.6</td>
<td>71.4</td>
<td>189.9</td>
</tr>
<tr>
<td>6</td>
<td>14.6</td>
<td>17.1</td>
<td>117.1</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>24.6 ± 3.6</td>
<td>34.6 ± 8.2</td>
<td>135.7 ± 11.6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21.5</td>
<td>22.6</td>
<td>105.1</td>
</tr>
<tr>
<td>2</td>
<td>16.6</td>
<td>18.7</td>
<td>112.7</td>
</tr>
<tr>
<td>3</td>
<td>36.4</td>
<td>33.5</td>
<td>92.0</td>
</tr>
<tr>
<td>4</td>
<td>12.4</td>
<td>11.9</td>
<td>96.0</td>
</tr>
<tr>
<td>5</td>
<td>22.3</td>
<td>21.6</td>
<td>96.9</td>
</tr>
<tr>
<td>6</td>
<td>34.1</td>
<td>36.2</td>
<td>106.2</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>23.9 ± 3.9</td>
<td>24.1 ± 3.7</td>
<td>101.5 ± 3.2</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with control rabbits (Student’s t-test).

*Figure 7.* (A) Histologic change in the ONH of a rabbit approximately 1 month after electrical stimulation. The optic cup was enlarged in the strongly stimulated eye and slight gliosis was observed in the prelaminar region. (B) Higher magnification of the square in (A). H&E staining; bar, 1 mm.
gesting that this technique could be used to produce an animal model of NTG. However, further study is required to formulate the optimal conditions for this model and its advancement for the development of new treatments for NTG.

References