Stimulation of the Cervical Sympathetic Nerves Increases Intraocular Pressure

Juana Gallar* and John H. K. Liu†

**Purpose.** To test the hypothesis that a moderate electrical stimulation of the cervical sympathetic nerves in rabbits can increase intraocular pressure (IOP).

**Methods.** Electrical stimulations of the cervical sympathetic nerves were performed in anesthetized and conscious rabbits. Intraocular pressure, pupil size, and concentrations of aqueous humor components were monitored.

**Results.** In urethane-anesthetized rabbits, stimulations of 5 V and 1 ms at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr caused a short inhibition of IOP decrease and a prolonged mydriasis. Concentrations of norepinephrine (NE), neuropeptide Y (NPY), and cyclic AMP (cAMP) in aqueous humor were elevated. Aqueous humor protein concentration was not changed. In rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital, electrical stimulations with the same parameters caused prolonged increases in IOP and pupil size. Aqueous humor NE and cAMP concentrations increased, while NPY and protein concentrations did not change. When the stimulations were set at 5 Hz for 3 hr under this anesthesia, the increase of IOP and mydriasis persisted. However, only the NE concentration increased. In conscious rabbits, stimulations of 5 V and 1 ms at either 5 Hz or 20 Hz were delivered from a portable stimulator for 4 hr, starting 2 hr before the onset of the dark. Stimulations at 5 Hz caused an increase in IOP in the light phase. The circadian IOP elevation in the dark phase persisted. When 20 Hz was used, a transient fall in IOP was observed, and the circadian IOP elevation was eliminated. Aqueous humor NE concentration doubled in conscious rabbits receiving electrical stimulations at 5 Hz for 1 hr.

**Conclusions.** A moderate electrical stimulation of the cervical sympathetic nerves can increase IOP in anesthetized rabbits and in conscious rabbits in the light phase. Invest Ophthalmol Vis Sci. 1993;34:596–605.

Intraocular pressure (IOP) can be regulated by many endogenous factors. The importance of each factor in different species may vary significantly. Among the many endogenous factors, the influence of sympathetic neural activities on IOP in rabbits has been extensively explored. The studies usually employed one of two approaches; an interruption of the sympathetic transmission or an electrical stimulation of the cervical sympathetic trunk.

An interruption of the sympathetic input to the rabbit eye was accomplished either by removing a sec-
tion of the cervical sympathetic trunk or by a superior cervical ganglionectomy. Rabbits with these surgical procedures had little circadian elevation of IOP in the dark phase.\textsuperscript{5,6} The circadian elevation of norepinephrine (NE), the postganglionic neurotransmitter, in aqueous humor in the dark phase disappeared.\textsuperscript{7,8} When the release of NE from the sympathetic nerves was suppressed by apraclonidine, the circadian IOP elevation was eliminated.\textsuperscript{9} These observations together indicate that endogenous activation of the ocular sympathetic nerves and the subsequent release of NE would cause an increase of IOP.

However, it has been documented throughout the past several decades that electrical stimulations of the cervical sympathetic nerves in anesthetized rabbits caused a decrease in IOP.\textsuperscript{10-15} This decrease of IOP also appeared in conscious rabbits.\textsuperscript{16} Electrical stimulations in these studies were performed in general using a high frequency (above 9 Hz), and the IOP was usually followed for only a relatively short time (minutes to 1 hr).

The circadian IOP elevation and the results from electrical stimulations highlighted the controversy about the ocular sympathetic role on rabbit IOP. In an attempt to reconcile different experimental results, we previously suspected that a moderate electrical stimulation of the cervical sympathetic nerves would increase IOP in rabbits.\textsuperscript{17} In the current study, we made a series of experiments employing electrical stimulations of the cervical sympathetic trunk at various frequencies in anesthetized rabbits. The changes in IOP, pupil size, cardiovascular functions, and aqueous humor components were measured. Electrical stimulations at selected frequencies were then performed in conscious rabbits using a portable stimulator. The effects on IOP, pupil size, and aqueous humor components were documented.

**METHODS**

Male New Zealand albino rabbits with body weights of 3.0-4.0 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research.

**Electrical Stimulation in Anesthetized Rabbits**

Electrical stimulations of the cervical sympathetic nerves were performed in three groups of ten rabbits anesthetized with urethane or a combination of ketamine, chlorpromazine, and pentobarbital. Previous studies with electrical stimulations in rabbits used two kinds of anesthesia: urethane\textsuperscript{12-15} and ultra-short- to short-acting barbiturates.\textsuperscript{11,12,15} We found that pentobarbital alone would not maintain a deep anesthesia for the 4-5 hr needed in the present study, and a combination of ketamine, chlorpromazine, and pentobarbital gave satisfactory results.

Rabbits in the first group were anesthetized with an IV injection of urethane (2.5 g/kg; Sigma, St. Louis, MO). Urethane was dissolved in sterile saline (25%) and given via the ear vein. Rabbits in the other two groups were anesthetized with IM injections of ketamine HCl (67 mg/kg; Vetalar; Aveco, Fort Dodge, IA) and chlorpromazine HCl (8 mg/kg; Rugby Laboratories, Long Island, NY) and an IV injection of pentobarbital sodium (15 mg/kg; Nembutal; Abbott Laboratories, North Chicago, IL). Supplemental urethane or pentobarbital was given during the experiment to maintain a stable anesthesia.

The cervical area of the anesthetized rabbit was exposed surgically. The preganglionic sympathetic trunk on one side was isolated, and a pair of silver electrodes was placed according to the description of Belmonte et al.\textsuperscript{18} The electrodes were wired to a Grass S48D stimulator (Quincy, MA) coupled to a Grass SIU5 isolation unit. A mydriatic response to a brief electrical stimulation (5V, 1 ms, and 20 Hz for a few seconds) was conducted to verify the circuit. The subcutaneous tissues and skin were closed by loose sutures. The ear artery on the operative side and the femoral artery were cannulated. Cannulae containing heparinized saline were connected to a Gould P23 ID pressure transducer (Oxnard, CA) and a Grass 79 polygraph.

**Physiologic Measurements**

The rabbit was placed in a prone position. After a waiting period of 30 min, IOP and pupil size in both eyes, ear and systemic blood pressures, and heart rate were measured. These values would be referred to as the initial values. IOP was measured with a pneumotonometer probe (Digilab, Cambridge, MA) attached to a pressure transducer and the polygraph. Proparacaine 0.1% (a fivefold dilution of 0.5% ophthaine; Squibb, Princeton, NJ) was given as a local anesthetic before tonometry if necessary. Pupillary diameter was measured using a ruler under constant illumination. Systemic and ear blood pressures were measured via the cannulae to the femoral artery and the ear artery. Heart rate was determined by the arterial pulse.

In urethane-anesthetized rabbits, stimulations of 5 V and 1 ms were delivered at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr. In the first group of ketamine-chlorpromazine-pentobarbital-anesthetized rabbits, electrical stimulations with the same parameters were performed. In the second group, stimulations of 5V and 1 ms were delivered at 5 Hz for 3 hr. Intraocular pressure, pupil size, systemic and ear blood pressures, and heart rate were measured every 15 min for 3 hr. Additional measurements were taken at 5 min after the beginning of the stimulation and at 5 min after the change of stimulation frequency.
Assay of Aqueous Humor Components

Rabbits were killed after the 3-hr stimulation by an i.v. injection of overdose pentobarbital. Aqueous humor was collected immediately from both eyes by paracentesis, and divided into one 100-μl and three 50-μl samples. Samples were frozen at −70°C. Aqueous humor was collected while the electrical stimulation was still on, because the blood-aqueous barrier could be damaged by the discontinuation of the electrical stimulation.19

Norepinephrine in the 100-μl aqueous humor sample was extracted and quantified by HPLC-electrochemical detection.20 Reagents for the assay were obtained from Waters (Milford, MA) and ESA (Bedford, MA). Neuropeptide Y in the 50-μl aqueous humor samples was determined using a RIA kit purchased from Peninsula Laboratories (Belmont, CA). Cyclic AMP in the 50-μl aqueous humor samples was determined using an assay kit from Advanced Magnetics (Cambridge, MA). Protein content in the 50-μl aqueous humor samples was determined by the Lowry method.

Electrical Stimulation in Conscious Rabbits

Ten rabbits were entrained in a daily 12 hr/12 hr light-dark environment7 for 3 weeks. The light period was from midnight to noon. A circadian elevation of IOP between 10:00 AM and 2:00 PM in each rabbit was verified. In the light phase, a stimulation device was surgically implanted around the cervical sympathetic trunk.18 Rabbits were anesthetized with ketamine, chlorpromazine, and pentobarbital, as described previously. The electrode wires were externalized to the back of the neck.18 Postoperatively, rabbits were allowed to recuperate in the same light-dark cycle. The IOP at 10:00 AM and 2:00 PM on the postoperative day 3 were measured. Successful preparations were identified by the appearance of the circadian IOP elevation, which indicated the sympathetic nerves were not damaged by surgery nor by the movement of the electrodes.

Electrical stimulations of the cervical sympathetic nerves were performed using a portable stimulator containing a 9-V battery. The stimulator delivered continuous trains of 5 V and 1 ms electrical pulses at selected frequencies. The amplitude, duration, and frequency of the stimulation were set using a Tektronix 2205 oscilloscope (Beaverton, Oregon). The electrode wires on the back of the neck were connected to the stimulator secured in a jacket worn by the rabbit (Harvard Apparatus, Natick, MA). Stimulation of 5 V and 1 ms were applied at 2.5 Hz for 4 hr (10:00 AM–2:00 PM) on the postoperative day 3 were measured. Successful preparations were identified by the appearance of the circadian IOP elevation, which indicated the sympathetic nerves were not damaged by surgery nor by the movement of the electrodes.

On the last day, another experiment with electrical stimulations at 5 Hz was performed on these rabbits for 1 hr (10:00 AM–11:00 AM). Rabbits were killed after the measurement at 11:00 AM. Aqueous humor was collected from both eyes, divided, and frozen at −70°C. The electrical stimulation was then turned off. Concentrations of aqueous humor NE, NPY, cAMP, and protein were determined.

Statistical Analysis

Data would be presented as mean±SEM. In anesthetized rabbits, comparisons of changes in IOP and pupil size were made between the two eyes. Blood pressures and heart rate were compared with the initial values. In conscious rabbits, IOP and pupil size were compared with values at 10:00 AM. Comparisons of aqueous humor components were made between the two eyes. The paired t-test was used in all cases. A difference of \( P < 0.05 \) was regarded to be statistically significant.

RESULTS

Rabbits Anesthetized with Urethane

Under urethane anesthesia, the initial IOP was 19.5 ± 0.9 mmHg \( (n = 10) \) in the stimulated eye and 20.8

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\text{FIGURE 1. Change in IOP after electrical stimulation of the cervical sympathetic nerves in urethane-anesthetized rabbits (n = 10). Stimulations of 5 V and 1 ms were applied at 2.5 Hz for 4 hr (10:00 AM–2:00 PM) on the postoperative day 3 were measured. Successful preparations were identified by the appearance of the circadian IOP elevation, which indicated the sympathetic nerves were not damaged by surgery nor by the movement of the electrodes.}
\]

\[ \text{FIGURE 1. Change in IOP after electrical stimulation of the cervical sympathetic nerves in urethane-anesthetized rabbits (n = 10). Stimulations of 5 V and 1 ms were applied at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr. Filled squares, the stimulated eye. Open squares, the contralateral eye: *, P < 0.05, **, P < 0.01.} \]
Sympathetic Stimulation Increases IOP

FIGURE 2. Change in pupil size after electrical stimulation of the cervical sympathetic nerves in urethane-anesthetized rabbits (n = 10). Stimulations of 5 V and 1 ms were applied at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr. Filled squares, the stimulated eye. Open squares, the contralateral eye; *, P < 0.05, **, P < 0.01.

± 0.8 mmHg in the contralateral eye. During the electrical stimulation, IOP decreased gradually in both eyes. The changes in IOP from the initial values were plotted in Figure 1. A difference in the rate of the IOP fall between the two eyes was noticed. There was little decrease of IOP in the stimulated eye when the electrical stimulations were performed at 2.5 Hz. A sharp drop of IOP occurred at 5 min after the stimulation frequency was increased to 20 Hz. Electrical stimulations at 2.5 and 20 Hz caused mydriasis; a larger response was seen with 20-Hz stimulation (Fig. 2).

A continuous decrease in systemic blood pressure occurred. In the 3-hr stimulation, the systolic blood pressure fell from 134 ± 5 mmHg to 90 ± 7 mmHg and the diastolic blood pressure fell from 85 ± 4 mmHg to 60 ± 6 mmHg. Heart rate remained in the range of 300-310 beats/min. Recording of the ear blood pressure was difficult because of vasoconstriction and was only successful in three out of ten rabbits.

After 3 hr of electrical stimulations, there were significant increases in aqueous humor NE, NPY, and cAMP concentrations (Table 1). The total aqueous humor proteins in the two eyes were indifferent.

Rabbits Anesthetized with Ketamine, Chlorpromazine, and Pentobarbital

In the two groups of rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital, the initial IOPs were between 14.2 ± 0.4 mmHg and 14.7 ± 0.3 mmHg. Throughout the electrical stimulation, IOP and pupil size in the contralateral eyes were stable. The differences in the changes in IOP and pupillary diameter (from the initial values) between the two eyes are shown in Figures 3 and 4. In the group that received electrical stimulations at 2.5–20 Hz, there was a frequency-dependent increase in IOP. Pupil size in

FIGURE 3. Difference in the change of IOP between the two eyes during electrical stimulation of the cervical sympathetic nerves in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital (n = 10). Open squares, stimulations of 5V and 1 ms at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr. Filled squares, stimulations of 5V and 1 ms at 5 Hz for 3 hr; *, P < 0.05; **, P < 0.01.

TABLE 1. Concentrations of Aqueous Humor Components After Electrical Stimulations of the Cervical Sympathetic Nerves in Urethane-Anesthetized Rabbits*

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Control Eye</th>
<th>Stimulated Eye</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>pg/ml</td>
<td>763 ± 100</td>
<td>6938 ± 1736</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NPY</td>
<td>pg/ml</td>
<td>14 ± 5</td>
<td>392 ± 106</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>cAMP</td>
<td>pmol/ml</td>
<td>43.1 ± 5.5</td>
<td>64.6 ± 7.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>mg/ml</td>
<td>0.621 ± 0.064</td>
<td>0.689 ± 0.099</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Rabbits were anesthetized with 2.5 g/kg urethane. Stimulations of 5 V and 1 ms were applied at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr.
† Paired t-test between the two eyes.
Values are mean ± standard error of the mean (n = 10).
FIGURE 4. Difference in the change of pupil size between the two eyes during electrical stimulation of the cervical sympathetic nerves in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital (n = 10). Open squares, stimulations of 5V and 1 ms at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr. Filled squares, stimulations of 5V and 1 ms at 5 Hz for 3 hr; *, P < 0.05; **, P < 0.01.

the stimulated eye did not increase with 2.5-Hz stimulation, but did with 20-Hz stimulation. In the group that received stimulations at 5 Hz for 3 hr, there was a consistent increase in IOP in the stimulated eye. The small increase in pupillary diameter was statistically significant at some time points.

The systemic blood pressure was relatively stable during the experimental period. The systolic pressure was between 88 ± 3 mmHg and 94 ± 3 mmHg, and the diastolic pressure was between 63 ± 3 mmHg and 72 ± 3 mmHg. Fluctuations occurred, in part, because of the supplemental injections of pentobarbital. Ear blood pressure fluctuated in general with the systemic blood pressure, except for a transient increase at 5 min after the beginning of the electrical stimulations. With 2.5-Hz stimulation, the increases of systolic and diastolic pressures were 14 ± 3 mmHg and 8 ± 2 mmHg (n = 5), respectively. With 5-Hz stimulation, the increases were 7 ± 1 mmHg and 7 ± 1 mmHg (n = 10). At other time points, no distinct pattern in the change of the ear blood pressure from the systemic blood pressure could be identified. The systolic pressure of the ear artery was between 86 ± 3 mmHg and 97 ± 5 mmHg. The diastolic pressure was between 63 ± 3 mmHg and 74 ± 3 mmHg. Heart rate decreased gradually during the electrical stimulations at 20 Hz. When the stimulation was performed at 5 Hz, the bradycardia did not appear until the last 30 min.

After the electrical stimulation of 2.5–20 Hz, there was a significant increase of NE and cAMP concentrations in the aqueous humor (Table 2). Stimulations at 5 Hz caused only an increase of NE concentration (Table 3).

Conscious Rabbits

The implantation of electrodes was successful in eight rabbits. The postoperative circadian IOP elevation was 4.2 ± 0.8 mmHg on the operated side and 4.6 ± 0.9 mmHg on the contralateral side. The preoperative circadian IOP elevation was 5.4 ± 0.7 mmHg on the operative side and 5.4 ± 0.5 mmHg on the contralateral side.

Changes in IOP in various experimental conditions are summarized in Table 4. When the electrical stimulations were performed at 5 Hz, an increase of IOP was observed at 11:00 AM and 12:00 PM. The circadian IOP elevation in the dark persisted. When the stimulations at 20 Hz were used, there was an IOP decrease at 10:10 AM. The circadian IOP elevation in the dark disappeared in the stimulated eye. There were decreases in IOP in the contralateral eye at 10:10 AM and 11:00 AM. Pupil size increased by both the 5-Hz and 20-Hz stimulations (Table 5). The second stimulations at 5 Hz caused similar responses in IOP and pupil size at 11:00 AM (Tables 4 and 5).

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Control Eye</th>
<th>Stimulated Eye</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>pg/ml</td>
<td>894 ± 228</td>
<td>9734 ± 4471</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NPY</td>
<td>pg/ml</td>
<td>19 ± 8</td>
<td>35 ± 9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>cAMP</td>
<td>pmol/ml</td>
<td>34.3 ± 4.2</td>
<td>45.6 ± 4.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>mg/ml</td>
<td>0.493 ± 0.062</td>
<td>0.481 ± 0.054</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Rabbits were anesthetized with intramuscular ketamine (67 mg/kg), intramuscular chlorpromazine (8 mg/ml), and intravenous pentobarbital (15 mg/ml). Stimulations of 5 V and 1 ms were applied at 2.5 Hz for 1 hr and then at 20 Hz for 2 hr.
† Paired t-test between the two eyes.

Values are mean ± standard error of the mean (n = 8–10).
TABLE 3. Concentrations of Aqueous Humor Components After Electrical Stimulations of the Cervical Sympathetic Nerves at 5 Hz in Rabbits Anesthetized With Ketamine, Chlorpromazine, and Pentobarbital*

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Control Eye</th>
<th>Stimulated Eye</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>pg/ml</td>
<td>568 ± 139</td>
<td>1671 ± 165</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NPY</td>
<td>pg/ml</td>
<td>26 ± 7</td>
<td>23 ± 8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>cAMP</td>
<td>pmol/ml</td>
<td>29.6 ± 3.2</td>
<td>30.6 ± 3.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>mg/ml</td>
<td>0.496 ± 0.046</td>
<td>0.429 ± 0.035</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Anesthesia was the same as in Table 2. Stimulations of 5 V and 1 ms were applied at 5 Hz for 3 hr.
† Paired t-test between the two eyes. Values are mean ± standard error of the mean (n = 10).

Aqueous humor was collected at 11:00 AM from the rabbits that received the second electrical stimulations at 5 Hz. There was a onefold increase of aqueous humor NE concentration and no change in the concentrations of aqueous humor NPY, cAMP, and protein (Table 6).

DISCUSSION

Our working hypothesis was that a moderate electrical stimulation of the cervical sympathetic nerves can increase IOP in rabbits. Stimulations with a moderate-high voltage at a high frequency would decrease IOP in rabbits, which was reported by many laboratories.12-15 In cats and dogs, such electrical stimulations might cause a transient increase of IOP before the decrease of IOP.12,21 This IOP increase was probably due to the contraction of the extraocular muscles.22 The IOP response to electrical stimulations at a low frequency has not been documented.

The cervical sympathetic trunk is composed of mixed nerves in different diameters, derived from several spinal cord segments.23 When the voltage of stimulation on the nerve is low, certain nerves would not be depolarized for neural transmission.24 Therefore, the voltage of electrical stimulation can be lowered to activate only a portion of the sympathetic nerves. However, surgical placements of the electrodes would produce variable resistances between the electrodes and the nerves. Data analysis would be difficult without knowing the precise voltage on the nerves.

Our approach was to vary the stimulation frequency while using a moderate voltage of 5 V. The pattern of the neurotransmitter release from the sympathetic nerves may depend on the frequency of stimulation.25 We selected two near-physiologic frequencies (2.5 and 5 Hz) and a supramaximal frequency (20 Hz).26-27 The concentrations of NE and NPY, the co-transmitter,28 in aqueous humor were measured. Our assumption was that a large change in these aqueous components would suggest a functional change in the sympathetic nerves.

TABLE 4. Changes in IOP (mmHg) During Electrical Stimulations of the Cervical Sympathetic Nerves in Conscious Rabbits*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time</th>
<th>10:10 AM</th>
<th>11 AM</th>
<th>Noon</th>
<th>2 PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated eye</td>
<td>Pre-operative</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.4 ± 0.7†</td>
</tr>
<tr>
<td></td>
<td>Post-operative</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.2 ± 0.8†</td>
</tr>
<tr>
<td></td>
<td>5 Hz</td>
<td>-1.0 ± 0.7</td>
<td>5.3 ± 1.7†</td>
<td>3.2 ± 1.5‡</td>
<td>3.9 ± 1.5‡</td>
</tr>
<tr>
<td></td>
<td>20 Hz</td>
<td>-5.4 ± 0.9†</td>
<td>-1.3 ± 0.9</td>
<td>-1.5 ± 1.1</td>
<td>-0.3 ± 1.1</td>
</tr>
<tr>
<td>Contra eye</td>
<td>Pre-operative</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.4 ± 0.5†</td>
</tr>
<tr>
<td></td>
<td>Post-operative</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.6 ± 0.9†</td>
</tr>
<tr>
<td></td>
<td>5 Hz</td>
<td>1.9 ± 1.5</td>
<td>1.0 ± 0.7</td>
<td>-0.2 ± 0.8</td>
<td>6.3 ± 1.4†</td>
</tr>
<tr>
<td></td>
<td>20 Hz</td>
<td>-1.0 ± 0.5‡</td>
<td>-2.2 ± 0.7†</td>
<td>-0.5 ± 0.7</td>
<td>1.9 ± 1.0‡</td>
</tr>
<tr>
<td></td>
<td>5 Hz</td>
<td>0.1 ± 0.6</td>
<td>0.9 ± 1.2</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* The change from the value at 10 AM. Stimulations of 5 V and 1 ms were applied from 10 AM. The onset of dark was at noon.
† P < 0.01.
‡ P < 0.05, paired t-test, compared with the values at 10 AM.
Values are mean ± standard error of the mean (n = 8).
Initial IOPs were 18.9 ± 0.9 mmHg to 22.4 ± 0.7 mmHg.
humor components would reflect a high sympathetic activity. Measurement of cAMP concentration was included because the cAMP in aqueous humor may have a direct effect on IOP, as well as reflect the biological activities in the anterior segment. The synthesis of cAMP in rabbit ocular tissues would also be modulated by NE and NPY. At 5 Hz is close to the physiologic condition. The mydriatic response was consistent. This frequency-dependent mydriasis was probably caused by a single mechanism: the contraction of the radial muscle. The difference in IOP responses in various experimental conditions, however, indicated that more than one mechanism was involved. With the electrical stimulations at 20 Hz in urethane-anesthetized rabbits, there was a sharp drop of IOP at 5 min into the stimulation. A similar acute IOP decrease was observed in many previous studies. This sharp IOP decrease was probably due to the reduction of ocular blood volume by the severe vasoconstriction at high frequency.

TABLE 6. Concentrations of Aqueous Humor Components After Electrical Stimulations of the Cervical Sympathetic Nerves in Conscious Rabbits

<table>
<thead>
<tr>
<th>Component</th>
<th>Unit</th>
<th>Control Eye</th>
<th>Stimulated Eye</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>pg/ml</td>
<td>566 ± 123</td>
<td>1126 ± 164</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NPY</td>
<td>pg/ml</td>
<td>50 ± 31</td>
<td>128 ± 33</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>cAMP</td>
<td>pmol/ml</td>
<td>33.2 ± 4.2</td>
<td>34.5 ± 5.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>ng/ml</td>
<td>0.623 ± 0.045</td>
<td>0.635 ± 0.081</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Stimulations of 5 V and 1 ms at 5 Hz were applied from 10 AM. Aqueous humor was collected at 11 AM.
† Paired t-test between the two eyes. Values are mean ± standard error of the mean (n = 8).
This vasoconstriction may be caused by the actions of NE and NPY, although other unidentified mediators may also be involved.

The IOP responses to the electrical stimulations at 2.5–20 Hz in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital were different from those in rabbits anesthetized with urethane. In urethane-anesthetized rabbits, an inhibition of IOP decrease occurred during the stimulations at 2.5 Hz, but not at 20 Hz. In rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital, there were IOP increases with the stimulations at 2.5 and 20 Hz. High frequency produced larger IOP increase. The mechanism causing the difference in IOP response at the high frequency was not clear. The lack of the co-release of NPY from the sympathetic nerves in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital may be related to the IOP increase. An absence of NPY release in conjunction with the release of NE from the splenic blood vessels by tyramine was observed in pigs anesthetized with ketamine, pentobarbital, and pancuronium. This IOP increase may also be related to the cardiovascular functions under this anesthesia. Our data showed a low initial IOP and blood pressure. Pentobarbital is known to decrease blood pressure and IOP. Chlorpromazine can also decrease IOP and the blood pressure at the dosage used. The cannulation and the recording of ear blood pressure in these rabbits were easy, indicating no severe vasoconstriction. We assumed that the vascular tone in the ear would indicate the vascular tone inside the eye under our experimental conditions, because both vessels are innervated by the postganglionic sympathetic nerves from the superior cervical ganglion. In rabbits anesthetized with pentobarbital alone, electrical stimulations at both low and high frequencies would cause vasoconstriction. However, we only observed a transient increase of ear blood pressure (presumably due to vasoconstriction) at the beginning of the electrical stimulations. There was no increase of ear blood pressure when the stimulation frequency increased to 20 Hz. The vasoconstriction due to electrical stimulations in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital was probably not very effective in counteracting the increase of IOP. In addition, ketamine inhibits the reabsorption of NE to the postganglionic adrenergic neurons, which may enhance the action of NE at the synapse that consequently increases IOP.

From the study of aqueous humor flow during the circadian IOP elevation, it was established that the increase of IOP was at least partially due to the increase of aqueous flow by the sympathetic activation. An increase of outflow resistance may also be involved, as is supported by pharmacological evidence. Corresponding to this IOP increase, there was an increase of NE in the aqueous humor. Because there was a very significant increase of NE in aqueous humor after 2.5–20-Hz electrical stimulation in rabbits anesthetized with ketamine, chlorpromazine, and pentobarbital, we proposed that this IOP increase was due to an increase of aqueous flow and an increase of outflow resistance. The increase in IOP during electrical stimulation at 5 Hz was probably due to the same mechanisms. Although the vasoconstriction may still exist during electrical stimulations at these frequencies, the net effect was an IOP increase.

In conscious rabbits, the relatively large fluctuation of initial IOPs was thought to be due to the short recuperation time allowed after surgery and after each experiment. Electrical stimulation of the cervical sympathetic nerves at 5 Hz in these rabbits produced an increase of IOP at 11:00 AM and 12:00 PM. The increase of 3.2–5.3 mmHg was reproducible. This observation strongly supports our hypothesis that electrical stimulation of the cervical sympathetic nerves can increase IOP. When the stimulation was applied at 20 Hz, a classical sharp decrease of IOP was observed. Because the movement of the implanted electrodes was unpredictable, some of the electrical stimulation at high frequency may spread to the adjacent tissues and, subsequently, affect the ocular functions in the contralateral eye. This may explain the contralateral effects seen at the high frequency.

After 1 hr electrical stimulations at 5 Hz, the aqueous humor NE concentration doubled but the NPY and CAMP concentrations did not change. The major mediator for the IOP increase is probably the NE. This observation supports our current thinking that the neural release of NE causes an IOP increase in normal physiologic conditions. The postsynaptic intracellular pathway that causes the IOP increase is unclear. Because there is no change of cAMP concentration in the aqueous humor, the cAMP-mediated intracellular pathway is probably not involved. Perhaps, the phosphoinositide cascade is involved in the IOP elevation, as occurred in the sympathetic excitation-contraction coupling of the iris muscles.

It was shown in a previous study that a short IOP increase occurred in rabbits that received an IV injection of NE. Endogenous NE from the systemic sources is capable of increasing IOP in rabbits. Recent studies also indicate that aqueous humor NE plays a minimal role in causing the decrease of IOP in the "ganglionectomy effect." These observations and the results from the current study are in agreement with the positive effect of circulating catecholamines on aqueous humor flow in humans. The possible increase of outflow resistance by the neuronal NE in rabbits is unique. In humans, no change of outflow resistance was observed after topical application of NE. However, a decrease of outflow resistance oc-
curs after topical epinephrine.\textsuperscript{45} For the effects of catecholamines on human aqueous humor dynamics, the postsynaptic receptors in the signal transduction pathway is beta-adrenergic.\textsuperscript{5,45} In rabbits, the beta-adrenergic receptor plays only a limited role in the IOP elevation due to the endogenous release of neuronal NE.\textsuperscript{5}

We concluded that electrical stimulation of the cervical sympathetic nerves in laboratory rabbits can increase IOP. Compared with previous studies,\textsuperscript{10-16} the electrical stimulations at 5 Hz in the present study seem moderate and closer to normal physiologic condition. Based on the results in the conscious rabbits, we ascertain that immediately before the onset of dark, when the sympathetic tone is low, electrical stimulations of the cervical sympathetic nerves will cause an increase in IOP. A similar endogenous factor probably is active around the onset of dark and causes the circadian IOP elevation in rabbits.

**Key Words**
electrical stimulation, intraocular pressure, norepinephrine, rabbit, sympathetic nerve

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