Effects of Stimulation of the Ocular Sympathetic Nerves on IOP and Aqueous Humor Flow

Carlos Delmonte,* Stephen P. Bartels, John H. K. Liu, and Arthur H. Neufeld

Ocular sympathetic nerves were stimulated chronically in awake rabbits using electrodes unilaterally implanted on the cervical sympathetic trunk. IOP was measured by pneumatonometry and aqueous inflow was measured by fluorophotometry. In each animal, continuous trains of 1 msec pulses were delivered by means of a portable electrical stimulator. Experiments were spaced by 1 week recovery periods. Stimulation was varied over a range of amplitudes (5–15 V) and frequencies (3–12 Hz). Continuous sympathetic stimulation produced an immediate sharp decrease in IOP followed by a gradual rise to pre-stimulation values which were attained 60–90 min after onset. A rebound increase in IOP occurred when stimulation was terminated. The magnitude of the initial IOP drop, the delay in the return to pre-stimulation IOP, and the rebound rise in IOP subsequent to termination of electrical stimulation were proportional to the stimulation frequency. Maximal effects were observed at 12 Hz, and stimulation with 8–10 Hz for 180 min caused a sustained reduction in anterior chamber aqueous humor flow. Topical 2% phentolamine 1 hr before stimulation markedly reduced IOP and abolished the acute IOP changes observed in untreated stimulated animals. Topical 1% timolol did not affect either the initial IOP drop or the rebound; however, the IOP recovered during stimulation to values greater than pre-stimulation IOP. We conclude that in rabbits the β-adrenergic effect of prolonged sympathetic nerve stimulation is to decrease aqueous flow. Chronic electrical stimulation in awake animals provides an experimental model for studying the role of the ocular sympathetic nerves. Invest Ophthalmol Vis Sci 28:1649–1654, 1987

Adrenergic influences on aqueous humor dynamics and intraocular pressure (IOP) are documented1–3; however, the responsiveness of these parameters to adrenergic manipulations depends greatly on the experimental conditions and on the animal species employed. Noted differences in responsiveness may be due in part to the degree of efferent sympathetic activity. For example, stress, circadian rhythm or anesthesia alters release of endogenous transmitter and causes variation in background levels of activity upon which exogenous adrenergic influences interact.

Electrical stimulation of the cervical sympathetic nerves has been employed repeatedly to mimic the effects of physiological excitation of the ocular adrenergic fibers on aqueous humor dynamics and IOP. Henderson and Starling4 first noticed a decrease of IOP in anesthetized cats and dogs following sympathetic stimulation, an observation later confirmed by others in the rabbit.5–7 In an extensive study, Langham and Rosenthal8 reported that in the rabbit sympathetic stimulation for periods up to 30 min reduced IOP, aqueous humor formation and blood flow to the ciliary processes, whereas the drainage of aqueous humor remained unaffected. All these studies, however, were performed in anesthetized animals, using manometric measurement of IOP and thus were limited to short periods of time.

Recently, a technique has been described to perform prolonged stimulation of the cervical sympathetic nerve in unanesthetized animals using chronically implanted electrodes.9 We have applied this method to examine the effects of sustained electrical stimulation of the ocular sympathetic nerves during extended periods of time on IOP and aqueous humor flow. Also, the actions of α- and β-adrenergic antagonists on the IOP responses to adrenergic nerve stimulation have been explored.

Materials and Methods

Pigmented rabbits of both sexes weighing 2.5–3.5 kg were used. Experiments were performed according
Electroscope Implantation

Animals were anesthetized with ketamine (66 mg/kg) and xylazine (7 mg/kg) injected intramuscularly, followed by pentobarbital (15 mg/kg) intravenously. The preganglionic sympathetic trunk was carefully dissected and two stimulating electrodes were implanted on the nerve, according to the technique described by Belmonte et al.9

The electrode terminals were exteriorized at the back of the neck. Electrical stimulation was performed briefly at the end of surgery and the pupillary response checked in order to ascertain the integrity of the ocular sympathetic fibers. The animals were allowed to recover from surgery for 1 week. The pupillary response was provoked several days later, and only those rabbits that exhibited a clear mydriasis in response to electrical stimulation were used.

Electrical Stimulation

Postoperative rabbits were put into restraint boxes. Stimulation of the cervical sympathetic nerve was performed using a portable electrical stimulator that could deliver continuous trains of 0.5 to 1 msec pulses of 1 to 12 V at a frequency of 1 to 20 Hz.9 In some experiments a stimulator coupled to a stimulus isolation unit (Grass Instruments, Quincy, MA) was also used. Prior to the experiment, the stimulating voltage was adjusted to the value necessary to evoke a maximal pupillary response at the desired frequency. The stimulatory pulses were visualized on the screen of an oscilloscope to adjust accurately the amplitude and frequency of the stimulation.

After basal IOP readings were taken for at least 30 min, the stimulus was initiated and the pupillary response again checked. Stimulations were maintained for periods of 30–270 min depending on the experimental design. The animals were removed from the restraint box when long stimulation periods were used, but the stimulator remained connected to the electrodes and secured in a jacket worn by the rabbit.

IOP Measurements

IOP was measured with a pneumatonometer probe (Digilab Inc., Cambridge, MA) attached to a pressure transducer and a physiograph. The calibration of the tonometer was performed in intact eyes of anesthetized rabbits by the closed stopcock manometric method. Half percent proparacaine was used as local anesthetic before tonometry. At least three successive IOP readings were taken and the average was recorded for each measurement. Postoperative rabbits were acclimated to pneumatonometry by making several IOP measurements prior to beginning each experiment.

Fluorophotometry

Corneas were stained with topical fluorescein on the day prior to fluorophotometric studies. Concentrations of fluorescein in the cornea and anterior chamber were measured at 30 min intervals with a slit-lamp fluorophotometer.10 Anterior chamber aqueous humor flow rate was calculated using a clearance method based on that described by Brubaker.11 Corneal volume was assumed to be 75 µl and anterior chamber volume 250 µl.

Drugs

All concentrations refer to weight/volume of base. Ketamine was from Parke-Davis (Morris Plains, NJ), xylazine from Haver-Lockhart (Shawnee, KS), pentobarbital from Abbott Laboratories (North Chicago, IL), phentolamine mesylate was the generous gift of Ciba-Geigy Corp. (Summit, NJ) and timolol maleate was the generous gift of Merck, Sharp and Dohme (West Point, PA).

Results

Changes of IOP With Sympathetic Stimulation

Figure 1 shows the pattern of IOP variation resulting from the electrical stimulation of the cervical sympathetic nerve for 3 hr. Data were pooled from experiments in seven rabbits stimulated with continuous trains of 1 msec, 12 V pulses at 9 Hz on different days with a minimum interval of 4 days between trials. Initiation of the stimulus resulted in an immediate IOP drop of 4.4 mmHg concomitant with marked mydriasis, followed by slow recovery of IOP to control values, within 60 to 90 min after the onset of stimulation. Cessation of nerve stimulation re-
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resulted in an abrupt pressure rebound of 2.1 mmHg, followed by a gradual IOP decay that reached pre-stimulation levels in approximately 30 min.

The magnitude of the initial IOP drop immediately following initiation of the stimulus was proportional to the stimulatory frequency, as illustrated in Figure 2a. Maximal effects were observed with 12 Hz, which produced a mean ± SEM pressure decrease of 6.8 ± 1.5 mmHg (n = 10), compared with an IOP drop of 3.2 ± 0.8 mmHg (n = 10) when 3 Hz were applied. The time course of the subsequent IOP recovery during maintained stimulation was related to the frequency of stimulation (Fig. 2b). At lower stimulatory frequencies (3 and 6 Hz), mean control values recovered after 30 min, whereas, when higher frequencies were used, IOP still remained 2–3 mmHg under pre-stimulation levels at this time period. At the higher frequencies, IOP recovered to pre-stimulation values within 60 min (Fig. 2b) and remained normotensive for the next 2 hr. The magnitude of the IOP rebound following cessation of stimulation was also directly related to the stimulatory frequency (Fig. 2a) and was proportionally smaller for a given frequency than the corresponding initial IOP drop elicited by the onset of the stimulus.

Fig. 2. (a) Initial IOP drop and IOP rebound during chronic electrical stimulation with different frequencies in conscious rabbits. All data are means ± SEM (n = 8–13). (b) The recovery of IOP during electrical stimulation at different frequencies in conscious rabbits. ▽, 3 Hz; ◇, 6 Hz; △, 9 Hz; and ▽, 12 Hz. All points are means ± SEM (n = 5–11).

Effects of Sympathetic Stimulation on Anterior Chamber Aqueous Humor Flow

Aqueous humor flow rate was measured in both eyes of rabbits at baseline, control conditions and during a 4.5 hr unilateral stimulation of the cervical sympathetic nerves. The basal rates in both eyes of each animal (n = 13), when no stimulation was performed, were quite similar and no significant differences were found between the control (3.08 ± 0.19 μl/min) and the operated sides (3.18 ± 0.19 μl/min). Thus, implantation of the electrodes did not alter the basal aqueous humor flow rate.

Sympathetic stimulation for 3 hr at 8–10 Hz causes a relatively small reduction (12–19%) in aqueous humor flow rate of the stimulated side when compared with the contralateral, non-stimulated eye. The time course of this reduction in flow is shown in Figure 3. The flow rate was calculated at 30 min intervals throughout a 4.5 hr stimulation period, starting immediately after the onset of the stimulus. The consistently, statistically significant reduction of aqueous humor flow occurred as early as 120 min after beginning stimulation and persisted. Perhaps flow was decreased at earlier time points but the data does not permit resolution of these points. Thus, aqueous humor flow rate was reduced at a time during continuous stimulation when IOP had recovered to pre-stimulation values.

Fig. 3. Effects on aqueous flow with chronic electrical stimulation of the cervical sympathetic nerves in conscious rabbits. Electrical stimulation was started at time = 0. 8–10 Hz were used. All data are means ± SEM (n = 6–9). □ = contralateral control, ■ = electrically stimulated.
The Influences of Adrenergic Antagonists on IOP Responses to Sympathetic Stimulation

The effects of the α-adrenergic antagonist, phentolamine, and the β-adrenergic antagonist, timolol, were explored in separate series of experiments.

As depicted in Figure 4a, topical 2% phentolamine applied twice to one eye of non-operated rabbits produced a gradual decrease of IOP in the treated eye over a 2 hr period and also, to a lesser extent, in the non-treated, contralateral eye. Two hours after the administration of the drug, the IOP had fallen about 8 mmHg in the treated eye and about 4 mmHg in the contralateral eye. At 4 hr, low IOP values were still present in both eyes and at 7 hr, IOP had recovered only partially.

Electrical stimulation of the cervical sympathetic nerves was performed in rabbits pretreated unilaterally with 2% phentolamine in the stimulated eye 1 hr before the onset of stimulation. The IOP of phentolamine-treated, stimulated eyes was compared to the IOP of phentolamine-treated eyes of non-operated rabbits (Fig. 4b). The curve for stimulated eyes is not significantly different than that resulting from the administration of phentolamine alone. Thus, when phentolamine is present, the initial IOP drop and subsequent IOP recovery does not occur in response to electrical stimulation.

Timolol (1%) was administered bilaterally and followed 1 hr later by unilateral electrical stimulation of the cervical sympathetic nerves for 3 hr. In the presence of timolol, electrical stimulation caused the initial IOP to drop about 3 mmHg in 1 min. However, within 15–30 min, IOP recovered to pre-stimulation levels. The IOP continued to rise during the following hour of stimulation, attaining values that were higher than the pre-stimulation values and that persisted during the remaining stimulation time (Fig. 5). Interruption of the stimulus resulted in an acute IOP rebound and a gradual return to control values.

The hypertensive action of sympathetic stimulation in timolol-treated eyes is illustrated in Figure 5 by comparison to the effects of sympathetic stimulation on IOP in animals not treated with timolol. Figure 5 presents the IOP differences compared to pre-stimulation values of electrically stimulated eyes either in the absence or presence of timolol. In timolol-treated animals, the amplitude to which IOP recovers during electrical stimulation is significantly higher from 60–180 min. Thus, in the presence of timolol, eyes receiving continuous sympathetic stimulation become hypertensive.

Discussion

Chronic implantation of stimulating electrodes on the cervical sympathetic nerves does not appear to interrupt the spontaneous nervous traffic in the adrenergic nerve fibers to the eye. No atypical IOP, pupillary or vascular signs were observed on the operated side in the days following surgery that would have been suggestive of damage to the nerve. Thus, we assume that electrical stimulation activates the
ocular sympathetic fibers, resetting the spontaneous activity to the imposed stimulatory train frequency and reproducing experimentally the effects of a maintained sympathetic discharge on the eye.9

Sustained stimulation of the ocular sympathetic fibers evoked in our experiments a consistent response of the IOP. The decrease in IOP that followed onset of stimulation has been observed in anesthetized animals4 and has been attributed to reduction in blood volume resulting from vasoconstriction of the richly innervated uveal vascular bed.12 In monkeys, Casey13 estimated that the decrease of ocular volume that occurs when sympathetic nerves are stimulated at 10 Hz is about 5 μl. Measurements of blood flow changes with equivalent sympathetic stimulation in the rabbit indicate that such a volume decrease is the result of a 60–75% reduction in ocular blood flow.12,14 The increasing magnitudes of the initial drops in IOP with higher stimulatory frequencies reflect the well-known relationship between frequency of sympathetic discharge and degree of vasconstriction.9,14 Apparently the opposite effect, a sharp increase in IOP, occurs when the stimulus is interrupted, and the IOP rebounds. The smaller amplitude of this pressure rise, in comparison with the magnitude of the equivalent IOP decrease at the onset of the stimulation, probably reflects a degree of smooth muscle relaxation in the ocular vessels which occurs even in the presence of maintained adrenergic stimulation.

Continuous measurements of IOP during sympathetic stimulation in anesthetized animals performed by previous investigators indicate that IOP did not return to pre-stimulation levels after 30 min of sustained stimulation. Langham and Rosenthal8 reported that under these conditions, blood flow to the ciliary processes and aqueous humor production were steadily decreased while outflow resistance was not altered. These authors concluded that adrenergic stimulation produced a sustained lowering of ocular equilibrium pressure as a consequence of reduction in aqueous humor inflow.

Our data confirm the 30 min time point of the above studies and provide evidence that when longer stimulations are performed in unanesthetized animals, IOP returns to control values despite a sustained lowering of the aqueous humor flow rate. This implies that a decrease in outflow occurs over time as a result of the maintained adrenergic stimulation. Decreased outflow can occur because of increased outflow resistance or decreased outflow pressure. Patterson15 reported an increase in outflow facility (decreased outflow resistance) in the cocaine-pretreated anesthetized rabbit approximately 30 min after electrical stimulation of the sympathetic nerves. Perhaps, intrascleral vasoconstriction during sustained electrical stimulation decreases outflow by decreasing net outflow pressure.

The topical, unilateral administration of the α-adrenergic antagonist, phentolamine, lowered IOP both in the treated and the contralateral eyes, confirming previous data on the action of α-adrenergic antagonists on IOP.16-20 The drug also blocked the IOP effects of electrical stimulation of the sympathetic nerves, confirming that the immediate IOP decrease 1 min after onset of stimulation is due to vasocostriction via mostly α-adrenergic activity. However, the low IOP values produced by the drug may obscure other, more subtle actions resulting from the excitation of β-adrenergic receptors by endogenously released neurotransmitter. In the presence of phentolamine, IOP did not recover to control levels during sustained stimulation. Perhaps phentolamine also blocked an α-adrenergic-mediated decrease in outflow, ie, vasocostriction.

The electrically stimulated, endogenously released neurotransmitter, norepinephrine, clearly acts at β-adrenergic receptors. This is indicated by the results of the timolol experiments. In these experiments, the initial IOP drop is quickly followed by a rapid increase of IOP to values higher than pre-stimulation levels. Thus, timolol potentiates and enhances the recovery of IOP during continuous sympathetic stimulation, perhaps because timolol blocks β-adrenergic-mediated decrease in inflow and therefore potentiates the α-adrenergic-mediated decrease in outflow.

We hypothesize that prolonged stimulation of β-adrenergic receptors in rabbits reduces IOP by decreasing aqueous humor formation. Our findings can therefore be interpreted as a decrease in the outflow produced by α-adrenergic stimulation during prolonged sympathetic stimulation offset by decreased aqueous humor formation produced by β-adrenergic receptor stimulation. Prolonged, high-frequency nerve stimulation may give different results than acute nerve stimulation or use of topical agonists. However, in all these cases, the initial effect may be increased aqueous human formation which becomes decreased upon prolonged stimulation because of β-adrenergic receptor desensitization.21

The existence of a continuous adrenergic tone influencing IOP is controversial.3 Probably the amount of sympathetic activity reaching the eye varies widely and depends on environmental conditions, diurnal rhythm and the level of stress involved in the experimental manipulations. This could explain in part the variable effects produced by ocular drugs that influence the adrenergic system. In our experiments, electrical stimulation of sympathetic nerves excites simultaneously all adrenergic fibers directed to the eye and corresponding receptor sites. Therefore, the elec-
trical stimulation of the ocular sympathetic nerve is a useful method to minimize the variations in ocular adrenergic tone, because it clamps spontaneous nervous activity at an established level. This technique will be useful in evaluating the IOP responses to adrenergic drugs.

**Key words:** intraocular pressure, aqueous humor flow, sympathetic nerves, α-adrenergic antagonist, β-adrenergic antagonist, rabbit

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**References**


