References


Chronic Stimulation of Ocular Sympathetic Fibers in Unanesthetized Rabbits

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The goal of this study was to devise a technique to implant permanent electrodes in the cervical sympathetic trunk, to stimulate the ocular adrenergic fibers for periods of hours or days in awake, unrestrained rabbits. Electrodes were made of a silver wire soldered to a multistranded wire and enclosed in silicone. Two of these electrodes were wrapped around the preganglionic sympathetic nerve, their leads emerging through a hole in the back of the neck. Success of the procedure was confirmed by the mydriasis elicited by electrical stimulation of the nerve following surgery; threshold voltages for the pupillary response varied between 5-10 volts. In eight rabbits, suprathereshold sympathetic stimulation was performed on the following days by means of a portable stimulator using increasing frequencies (1, 3, 5, 8, and 10 Hz) during a 20-hr period. Dilation of the ipsilateral pupil and vasoconstriction in the ear, measured by the fall in temperature of the ear's surface, was observed as long as stimulation was maintained. Both effects were proportional to the frequency of stimulation. Maximal mydriasis was obtained at 8 Hz, whereas full vasoconstriction was elicited with 5 Hz. Intraocular pressure, measured in 10 rabbits with a Perkins tonometer at the end of a 24-hr stimulation period, did not differ from pre-stimulation values. It was concluded that chronic stimulation of the sympathetic nerve allows to maintain known levels of adrenergic activity in the eye, and may be a useful method to study the actions of the adrenergic system on various ocular functions in unanesthetized animals. Invest Ophthalmol Vis Sci 28:194-197, 1987

Electrical stimulation of the cervical sympathetic nerve has been frequently used to determine the influence of the adrenergic system on various ocular functions. For instance, acute stimulation of the sympathetic reduces intraocular pressure, blood flow through the uveal vessels, and aqueous humor production. However, little is known about the circumstances in which peripheral adrenergic mechanisms are physiologically activated in the eye, and what is the role played by continuous sympathetic activity, the sympathetic tone, in the maintenance of such functions. Experimental approaches to these questions have used surgical or chemical denervation or blocking drugs to abolish sympathetic effects. These drastic procedures totally suppress adrenergic activity, but, with denervation, the normal physiology of the target organ is altered.

The aim of our work was to devise a technique using chronic stimulation of the cervical sympathetic nerve that would allow us to adjust, for long periods of time, the degree of sympathetic activity that reaches the eye in awake, unrestrained animals. Preliminary results have been reported elsewhere.4

Materials and Methods. Electrodes: Implantable electrodes based on those described by Sweet and Bourassa1 were constructed. The electrodes consisted of a rectangle of 6 mm X 2 mm of silicone sheeting (slastic sheeting, 500-3, Dow Corning Corp. Medical Products, Midland, MI) in which a 0.125 mm diameter silver wire was pierced through two small holes and soldered to the lead wire. This lead wire was introduced within a silastic tube to cover the wire up to the soldering point with the silver filament. A second, thinner...
Surgical procedures: Adult pigmented and albino rabbits were anesthetized with intravenous sodium pentobarbitone (Nembutal, 30 mg/Kg). Animals were treated according to the ARVO Resolution on the Use of Animals in Research. The preganglionic sympathetic trunk was identified in the neck and carefully dissected for a 2 cm length using a stereomicroscope. Two electrodes were introduced and wrapped around the nerve trunk, closing the electrode borders with a 8-0 suture. The electrodes were further secured in place with sutures to the neighboring muscles; electrodes were separated from each other by a distance of 5–10 mm (Fig. 1D–F). Care was taken to maintain the nerve in its natural position without being twisted by the electrodes. The lead wires were carried subcutaneously to the back of the neck and passed to the outside through a hole in the skin. The integrity of the cervical sympathetic trunk was ascertained at the end of surgery by the induction of mydriasis with electrical stimulation (see Results).

Stimulation: Electrical stimulation was performed using 1 ms square pulses of variable amplitude (3–15 V) and frequency (1–15 Hz) delivered by an Ortec 4710 electrical stimulator (Ortec Inc., Oak Ridge, TN) connected to a stimulus isolation unit in acute trials, and by a portable stimulator when prolonged stimulation in the unrestrained animal was desired. This battery-driven portable stimulator produced continuous trains of 0.5–1 ms duration pulses at adjustable frequencies ranging from 0.1–20 Hz and amplitudes of 0–15 volts; due to its reduced dimensions (5 X 3 X 2 cm), the rabbits could carry the stimulator for several days in a bag attached to their backs without apparent discomfort.

Pupillary, intraocular pressure and ear temperature measurements: The rabbits were placed in restraint boxes during the measurement periods. Pupillary diameter was measured with a ruler under uniform illumination. Intraocular pressure (IOP) was determined using a Perkins hand-held tonometer (Clement Clark, London, England). Tonometric readings were converted to mm Hg using a curve constructed with data obtained from 21 cannulated rabbit eyes, in which IOP was measured with a transducer and with the tonometer and artificially varied between 10 and 50 mm Hg in 10 mm Hg steps. Topical 0.5 propacaine was administered as local anesthetic. Temperature of the ear was gauged with a thermistor probe (YSI model 427) attached with adhesive tape to the internal surface of the ear and connected to an electronic thermometer (YSI model 42TD) and to a polygraph for continuous paper recordings of temperature.

Results. Every day during the week that followed surgery, the pupil response to sympathetic stimulation was tested using 10 Hz, 1 ms pulses to determine the threshold voltage at which a distinct mydriasis could be obtained. Threshold values stabilized usually between 5 and 10 volts; stimulating amplitudes over 12–15 volts were often accompanied by overt muscle contractions in the neck due to current spread, and the animal showed signs of unrest and discomfort. Hence, rabbits with thresholds over 12 volts were discarded. With stimulating voltages 1–2 volts over threshold, stimulation could be maintained for hours or days without apparent manifestations of displeasure or changes in the feeding or resting behavior of the rabbits. Failures occurred in about 30% of the operated animals, and appeared to be due to mechanical damage of the nerve due to displacement of the electrode or to tight fitting; it is apparently necessary to leave enough lumen to allow initial slippage of the nerve and space for invading connective tissue. Successful experiments were performed in 10 rabbits that exhibited stable threshold voltages of 10 volts or less during the 2-month period of this study.

Figures 2 and 3 show the time course of pupil dilation...
and ear temperature during a continuous suprathreshold stimulation lasting for 20 hr. Data are mean values of six rabbits stimulated at increasing frequencies with a 4–8 day interval between each stimulation. Maximal levels of pupillary and vascular responses were attained 10 min after the beginning of stimulation, and both remained stabilized for the following 6 hours. At 20 hr, some return of basal values could be observed both in the pupillary response and the ear’s temperature. There was correlation between the magnitude of the pupillary and vascular responses and frequency of stimulation. Figure 4 depicts the mean temperature and pupil diameter values of eight rabbits measured every hour during 6 hr of stimulation at different frequencies. For the vessels, maximal effects were attained with frequencies of 3–5 Hz with a clear response occurring at 1 Hz. For the pupil, more gradual changes were observed with increasing stimulation frequencies; maximal effects appeared at 8 Hz.

Intraocular pressure was measured in ten rabbits before and at the end of 24-hr stimulation periods at 3, 5, and 10 Hz. No significant variations of IOP were found at any of the stimulus frequencies explored (initial IOP: 15.6 ± 0.3 mm Hg, mean ± S.E., n = 10; IOP after a 24-hr stimulation: 16.2 ± 0.6 mm Hg, mean ± S.E., n = 9).

**Discussion.** The technique described here is based on implanting permanent, wrapping electrodes on the preganglionic cervical sympathetic trunk to chronically stimulate ocular adrenergic fibers and to reproduce the effects of their natural activation. This technique seems to offer satisfactory results in terms of integrity of the nerve fibers, responsiveness during prolonged stimulation periods, and reproducibility of threshold voltages after several weeks.

Pupillary dilation and decreased ear temperature upon stimulation were the results of well-known adrenergic effects on the iris dilator muscle and vascular smooth muscle. Maximal effects at a given frequency were obtained with voltages slightly over threshold. At these voltages, recruitment of all the sympathetic neurons in the ganglion probably occurs, even if not all the preganglionic fibers are excited, because these have a high degree of convergence; each ganglion neuron receives, on average, synaptic contacts from a minimum of ten different preganglionic fibers. Thus, even if a small percentage of the preganglionic fibers are damaged as a consequence of surgery, the synchronous activation of the remaining fibers should be sufficient to depolarize virtually all neurons in the ganglion. Furthermore, antidromic invasion of the somata of the preganglionic neurons by each stimulus should reset their membrane potential and reduce to near zero their spontaneous activity.

We found the expected correlation between stimulus frequency and magnitude of the pupillary and vascular response. Full pupillary dilation and vasoconstriction were obtained at frequencies over 3 Hz. In the cat, the
average spontaneous activity of preganglionic sympathetic fibers is 1.4 impulses/sec, reaching maximal frequencies of 9 impulses/sec, probably for only short periods of time, during stress conditions, such as acute hemorrhage. Hence, we can assume that an artificially imposed train of electrical pulses, which evokes a synchronous discharge in practically all postganglionic neurons, releases large amounts of neurotransmitter, which will probably produce maximal effects on the target organ at relatively low frequencies. Acute stimulation of the sympathetic produces a reduction of IOP in the anesthetized rabbit, but did not vary it substantially after 24 hr of stimulation in the awake animal. IOP is the final result of a complex balance between aqueous humor production and outflow, vascular resistance, and blood volume. Adrenergic influences on these parameters vary in magnitude and time course, and may produce antagonistic effects on IOP; thus, it is not surprising that drastic changes in basilar IOP were not observed after long-term stimulation.

The present experiments demonstrate that chronic stimulation of the cervical sympathetic in awake, unrestrained animals can be used at desired frequencies to reproduce known adrenergic effects in the eye. This technique will allow setting of the ocular sympathetic tone at pre-established levels, and will, therefore, be useful for expanding our knowledge of the role of the adrenergic system in the modulation of ocular functions, as well as the study of the action of drugs that mimic or interfere with ocular adrenergic effects.

Key words: adrenergic stimulation, ocular sympathetic, pupil, intraocular pressure, vasoconstriction

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References


Chorionic Gonadotropin Decreases Intraocular Pressure and Aqueous Humor Flow in Rabbit Eyes

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The effect of human chorionic gonadotropin (hCG) on the rabbit eye was studied. Intravitreal injections of hCG in albino rabbits provoked a reduction of intraocular pressure (IOP). Intravenous administration of hCG in single doses of 5,000-10,000 units in male pigmented rabbits caused a significant reduction in IOP from 1.5–5 hr after injection. When two successive intravenous doses of 5,000 units of hCG were given at 0 and 3 hr to pigmented rabbits, a significant reduction of net aqueous flow occurred, as measured by scanning fluorophotometry. These results indicate that the decrease in aqueous flow rate in the rabbit eye after administration of hCG can account for the reduction in IOP. Invest Ophthalmol Vis Sci 28:197–200, 1987

Gonadotropins are a class of glycoprotein hormones which include human chorionic gonadotropin (hCG), follicle stimulating hormone (FSH), and luteinizing hormone (LH). Each of these is comprised of two polypeptide subunits, an alpha and a beta chain. The alpha chains possess nearly identical peptide sequences,