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 basal 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

Acknowledgments. The authors wish to thank Mr. Alfonso Perez, Departamento de Fisiologia, Universidad de Alicante, and Mr. J. Fisher, FT Electrotek, 5386 Colter Drive, Kearns, UT 84118, for designing and building the portable stimulator; to Mr. S. Moya for technical assistance; and to Drs. A. Neufeld and C. Eyzaguirre for critical reading of the manuscript.

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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,
whereas the beta subunit confers the specificity of the hormones. These hormones are thought to exert their known biologic effect through activation of adenylate cyclase and the subsequent production of the second messenger, cAMP.

Human chorionic gonadotropin has been shown to lower intraocular pressure (IOP) in rabbits when given intramuscularly.1 Recent investigations in this laboratory have shown FSH/LH (pertonal) given intramuscularly to male or female rabbits and to oophorectomized female rabbits to decrease IOP. Substantial reductions in IOP occurred after intravitreal injection of hCG, whereas there was no decrease in IOP after single intravitreal injections of estrogen or progesterone.2 It is of interest to recall that there are clinical states in which increased levels of gonadotropins are associated with low IOP or hypotony. These include pregnancy3-5 and myotonic dystrophy,6-7 in which there are increases in the levels of hCG and FSH, respectively.

The present study was undertaken to assess the effect of intravenous hCG on aqueous humor flow rate and IOP, as well as to expand on previous work1-2 by more closely examining the effect of intravitreal hCG on IOP.

Materials and Methods. Male New Zealand albino rabbits were used for studying the effect of hCG on IOP after intravitreal injections. Male pigmented rabbits were used for studying the effect of intravenous hCG on IOP and aqueous humor flow rate. The experimental procedures employed here conform to the ARVO Resolution on the Use of Animals in Research. All animals weighed 2.0-2.5 kg, were fed ad lib, and were maintained in a 12 hr light/dark cycle.

Rabbit IOP was measured after placing 1 drop of proparacaine hydrochloride 0.5% (Ophthiotic) in each eye. Applanation pneumatonometry (Alcon, Fort Worth, TX) using a tonometer calibrated for rabbit eyes was used to determine IOP. Statistical analyses were conducted using the student's t-test.

Intravitreal injections: Rabbit eyes were anesthetized with 1 drop proparacaine hydrochloride 0.5% (Ophthetic). The hCG was diluted in an appropriate volume of sterile water to obtain concentrations of 2, 20, or 200 USP units per 10 μl. The right eye of each animal received 10 μl of the appropriate dose through a 30-gauge needle attached to a 50 μl Hamilton glass syringe. The contralateral left eye received an equal volume of sterile water and served as a control. There was no clinically observable anterior chamber or vitreous inflammation after the injections. Final molar concentrations of hCG were calculated to be 1.3 × 10^-9M, 1.3 × 10^-8M, and 1.3 × 10^-7M using 3 ml for total intraocular volume.8 Four rabbits were tested with each dose.

Intravenous injections: Five ml of a solution containing 1000-10,000 USP units of hCG was injected slowly (3 min) into a marginal ear vein in pigmented rabbits. Four rabbits were tested with each of five doses. An additional group of four rabbits received hCG 5,000 units 3 hr apart to obtain a prolonged effect on IOP. There were no obvious systemic side effects or changes in body temperature after the injections.

Measurements of aqueous flow: Measurements of the corneal and anterior chamber fluorescence were obtained using the Coherent Fluorotron Master Fluorophotometer (Palo Alto, CA). Treatment measurements were conducted at least 4 days after baseline measurements in the same animals.

At 1700 hr, on the day prior to scanning, 25 μl of a sterile 2.5% fluorescein solution was instilled into each eye every 5 min for a total of three doses with a Hamilton glass syringe. On the days of flow measurement, scans were obtained every 45 min starting at 1100 (18 hr after fluorescein instillation) and ending at 1500 hr. On the control day, each rabbit received an intravenous injection of 5 ml normal saline at 0900 and 1200. On the day of treatment, each rabbit received hCG 5,000 units intravenously (in 5 ml normal saline) twice, at 0900 and again at 1200 hr. This was done to insure relatively steady plasma levels of hCG. The rabbits were awake and unsedated during the scans, and were gently restrained using a modification of the rabbit holder described by Maurice and Singh.9

Since the measuring window on the Fluorotron is wider than the corneal thickness, the corrected corneal concentration (Cc) was calculated using the following formula:10

\[ Cc = Cm\{1 + 6.497 \times \exp(-5.146t)\}, \]

where Cm is the measured corneal concentration and t is the corneal thickness in millimeters (normal = 0.35 for the rabbit).

Aquous flow rates were calculated on both baseline and treatment days by the second method of Jones and Maurice.11

\[ f_o = A \times \frac{(CaVa + CeVc)}{Ca} \times 0.9, \]

where fo is the flow rate (in μl/min), A is the logarithmic slope of anterior chamber fluorescence reduction over time (calculated by method of least squares),12 Va is the anterior chamber volume (assumed to be 250 μl), Ca is the anterior chamber fluorescein concentration, Cc is the corrected corneal fluorescein concentration, Vc is the corneal volume (assumed to be 70 μl), and 0.9 assumes that 90% of the clearance of fluorescein from the anterior chamber is due to bulk flow while 10% is due to diffusion.11 Ca and Cc were both determined by extrapolating the appropriate decay curves back to time zero.12 The flow rate was calculated using six scans for each eye.
Results. Intravitreal hCG lowered IOP in male rabbits. The maximum decrease in IOP after intravitreal injection occurred at 24 hr. The average fall in IOP, comparing treated to control eyes, was 3.25 ± 1.25 mmHg (P = .04) after the maximum dose of 200 USP units. No statistically significant change in IOP was noted after the 20 and 2 USP unit intravitreal injections. The percentage change in outflow pressure was a decrease of 36% ± 13% from the contralateral baseline outflow pressure (Fig. 1).

Intravenous doses of 5,000, 7,500, and 10,000 USP units of hCG in pigmented rabbits caused a significant decrease in IOP compared to baseline. The maximum reduction in IOP occurred between 1.5 and 3 hr after injection. A peak reduction in outflow pressure of 65% was observed at 3 hr after a dose of 10,000 USP units hCG. During the interval of 2-6 hr after injections of 5000 UPS units hCG at 0 and 3 hr, there was a 39% ± 3% (P < .0005) reduction in outflow pressure. Also, during this interval there was a statistically significant decrease in outflow pressure (P < .05) with doses of 5,000, 7,500, and 10,000 USP units of hCG. No significant lowering of IOP was achieved with doses of 1,000 and 2,500 USP units of hCG (Fig. 2).

A total of 11 eyes of 8 male rabbits were acceptable, and were scanned on separate baseline and treatment days as described in the Materials and Methods section. The average decrease in aqueous flow was 25% ± 8% (N = 11) (P < .01). An alternate method to analyze the data was also used. The six eyes of the three rabbits with bilateral scans were viewed as three pairs. Each pair was averaged to obtain a single change in flow rate for each of these rabbits on the assumption that the two eyes of the same animal should behave similarly. These three values were then averaged with the five unilateral values and an average decrease in flow rate of 29% ± 7% (N = 8) (P < .005) was found. There was no statistical difference between the two methods of calculating percent change in flow before and after hCG. Figure 3 summarizes the results for each eye.

Discussion. The results of this study confirm that hCG decreases pressure after intravitreal or intravenous administration. It is interesting that no effect on IOP is observed after intravenous doses of 1,000 and 2,500 units of hCG, while a statistically significant and virtually equal reduction with 5,000, 7,500, and 10,000 USP units is effected; i.e., a true dose-dependent function was not established. A similar phenomenon was also observed with the intravitreal injections. This all or none or threshold type of response is similar to that found after cholera toxin. A possible explanation is that, once the active drugs get into the cell, an effect occurs. Finally, we measured aqueous flow rates in animals which received intravenous hCG and found that hCG causes a decrease in flow.

If hormones have a significant effect in the regulation of IOP, then there must be specific ocular target tissues which respond and can lead to steady state changes in IOP. Possible anatomic sites of regulation include the ciliary epithelia, subepithelial vascular plexus, trabecular meshwork, and outflow channels. Mathematical considerations also aid in the search for the loci of potential regulatory mechanisms of IOP. The relationship between the change in outflow pressure and resistance to outflow (the reciprocal of facility of outflow) and change in flow can be expressed as:

\[
\frac{\Delta(P_i - P_e)}{P_i - P_e} = \frac{\Delta R}{R} + \frac{\Delta F}{F},
\]

where \(\Delta(P_i - P_e)\) = change in outflow pressure, \(P_i = \) initial IOP, \(P_e = \) episcleral venous pressure, \(R = \) initial outflow resistance, \(\Delta R = \) change in outflow resistance, \(F = \) initial flow, and \(\Delta F = \) change in flow. Employing this simple formula, the experimental data is consonant with the idea that the reduction in aqueous flow rate alone can very largely account for the decrease in outflow pressure, because there was a 39% reduction in outflow pressure and a 29% decrease in flow rate during the period in which flow was measured.

The interest in the role of glycoproteins and peptides in IOP regulation evolved from an attempt to analyze the role of the adenylate cyclase receptor complex in the ciliary processes using nonadrenergic stimulation of the enzyme complex. hCG, shown to have an ocular hypotonic effect, is a hormone known to act via
INTRAVENOUS HCG

Fig. 2. Change in outflow pressure after single intravenous injections of five doses of hCG. During the interval of 2–6 hr after injection there was a statistically significant decrease in outflow pressure (P < .05) with doses of 5,000, 7,500, and 10,000 USP units of hCG.

Fig. 3. Aqueous flow measured on two separate days by scanning fluorophotometry. "Before hCG" represents baseline measurements over a 4 hr period for individual eyes. "After hCG" represents measurements made in the same eyes between hr 2 and 6 of the treatment day when doses of 5,000 units each were given on hr 0 and 3. Each eye served as its own control (n = 11).

the adenylate cyclase receptor complex in other tissues. Proof establishing a cyclase link in the eye has been difficult to demonstrate (Bausher L and Sears M, personal communication). Further work is required to establish a correlation between the functional effect of hCG and its subcellular target mechanism. A suggestion has been made that a mechanism modifying surface membrane glycoprotein residues on the ciliary epithelium could induce changes in aqueous humor inflow.16

Key words: aqueous humor flow, human chorionic gonadotropin, scanning fluorophotometer, rabbit, intraocular pressure

From the Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut. Supported in part by US Public Health Service Grant EY-00785-14 and EY-00237-24, The Connecticut Lions Eye Research Foundation, Inc., and Research to Prevent Blindness, Inc. Submitted for publication: November 7, 1985. Reprint requests: Joseph S. Elman, MD, Department of Ophthalmology and Visual Science, Yale University School of Medicine, 330 Cedar Street, P.O. Box 3333, New Haven, CT 06510.

References