Acute effect of epinephrine on aqueous humor formation in the timolol-treated normal eye as measured by fluorophotometry. ROBERT G. HIGGINS AND RICHARD F. BRUBAKER.

A double-blind, randomized, placebo-control study of the effect of epinephrine on the rate of aqueous formation in eyes pretreated with a beta-adrenergic blocking drug was carried out in 25 normal subjects with the use of fluorophotometry. All eyes were pretreated with timolol maleate before epinephrine or placebo was given. The effect of timolol maleate alone was to lower intraocular pressure and to reduce the rate of formation of aqueous humor. In the presence of timolol, aqueous formation was decreased further, approximately 7% more, in the epinephrine-treated eye as compared to the placebo-treated eye. This additional decrease in aqueous formation was statistically significant. No statistically significant difference was found in intraocular pressure or in tonographic C value between the epinephrine-treated and the placebo-treated eye. However, intraocular pressure was so low in both eyes that differences in intraocular pressure were not expected and tonographic tracings were difficult to interpret.

Epinephrine is an adrenergic agonist which is an effective ocular hypotensive agent in some subjects and is used as a topical treatment for glaucoma. Recently, it has been shown that the initial effect of epinephrine in the normal eye is to increase aqueous formation and increase aqueous humor outflow. The resultant effect, since the outflow effect is greater, is to decrease intraocular pressure. Timolol maleate is a beta-adrenergic blocker which is now used for treatment of chronic open-angle glaucoma. Topical application of this drug produces a rapid and prolonged lowering of intraocular pressure by decreasing production of aqueous humor.

The complex relationship between the adrenergic system of the eye and intraocular pressure in the human eye is not fully understood. Because epinephrine, a combined alpha and beta agonist, acts to increase aqueous formation and timolol maleate, a beta blocker, acts to decrease aqueous formation, we hypothesize that the initial action of beta agonists must be to stimulate the eye in some way to produce more aqueous humor. The purpose of this study was to see whether timolol was capable of blocking the early stimulatory effect of epinephrine on aqueous formation.

Methods. Twenty-five normal subjects were studied. An eye examination of each subject was carried out, consisting of best corrected visual acuity, external examination, slit-lamp examination, and tonometry. The admission criteria were that both eyes were normal (except for ametropia) and that intraocular pressure of the two eyes differed by 3 mm Hg or less.

A commercially prepared solution of 0.5% timolol maleate (Timoptic; Merck, Sharp & Dohme, West Point, Pa.) and 1% epinephrine hydrochloride (Epiprin; Allergan Pharmaceuticals, Irvine, Calif.) were used along with an identical-appearing placebo vehicle solution (Liquifilm; Allergan). The epinephrine/placebo bottle pairs were randomized by a hospital pharmacy to the right/left eye pairs of the subjects. The containers were identical and labelled as follows: "Subject 1, OD; Subject 1, OS; Subject 2, OD; Subject 2, OS; etc." The code was not known to the examiner until the data of all the subjects had been calculated. In determination of the effect of the drug, the treated eye of the subject was compared to the fellow placebo eye.

On the morning of the study, both eyes of the
subject were treated with 2 drops of timolol maleate (0.5%). One hour later, intraocular pressure was determined with the Perkins tonometer. Evaporated milk, rather than fluorescein, was used to delineate the area of corneal flattening in order to avoid premature staining of the cornea.

The volume of the anterior chamber was measured by the photogrammetric method of Johnson et al. The color of the iris was noted.

Two drops of epinephrine/placebo solution were instilled into each eye following tonometry. Thirty minutes later fluorescein iontophoresis was carried out in the central 4 mm of the cornea with an electrode of 10% fluorescein in 2% agar. A current of 200 µA was used for 7 sec, depositing 0.3 to 0.9 µg of fluorescein beneath the epithelium. The conjunctival sac was irrigated with balanced salt solution to remove any surface fluorescein.

Fluorescein concentration and mass were measured in the cornea and the anterior chamber immediately following iontophoresis and thereafter at 0.5, 2, and 7 hr with the fluorophotometer described by Brubaker and Coakes. The epinephrine/placebo solutions were instilled again at 2 hr. The intraocular pressure was remeasured at 7 hr.

The fluorophotometric data were used to calculate the anterior chamber mass elimination coefficient, Ke, and the rate of aqueous humor flow, F. These coefficients were determined by the nomographic method described by Coakes and Brubaker.

The two sided t test for paired samples (in a normal distribution) and the paired Wilcoxon test for nonnormal distributions were used to determine statistical significance. The accepted level of significance, α, was 0.05. All values listed were calculated by the paired Wilcoxon test; the paired t test was also performed and showed more significant value in every case.

Results. Table I outlines the results of each subject and illustrates the mean, S.D., and S.E. of each measured and calculated variable for the entire group. Aqueous humor flow was lower in the timolol/epinephrine eyes than in the timolol/placebo eyes (p = 0.04).* Also the anterior chamber elimination coefficient (Ke) was lower in timolol/epinephrine eyes (p = 0.05).* No other significant differences were noted between the two groups. (Subjects 2 and 12 were excluded from the statistical analysis because their additional intraocular pressures were so low, less than the reported lower limit of normal episcleral venous pressure, that the hypotony per se might directly alter the rate of ultrafiltration across intraocular vessels everywhere in the eye.)

Twenty of the subjects who participated in this experiment were also studied with a similar protocol and technique to measure the effect of epinephrine vs. placebo. These data allowed us to compare the results of timolol/placebo vs. placebo (Table I) and timolol/epinephrine vs. epinephrine alone (Table I) by comparing the same subject in one test to himself in the other test. 

Table I outlines the mean and S. D. of each variable of the timolol/placebo vs. placebo group. Very significant differences (p < 0.005)* were observed in initial intraocular pressure, anterior chamber elimination coefficient (Ke), and aqueous humor flow, with all values lower in the timolol/placebo eyes.

Table I outlines the differences between timolol/epinephrine vs. epinephrine alone. Very significant differences (p < 0.005)* were again found in initial intraocular pressure, anterior chamber elimination coefficient (Ke), and aqueous humor flow. A less significant difference (p < 0.025)* was observed in final intraocular pressure.

Table II summarizes the statistical comparisons of the data found in Table I. When compared to placebo, epinephrine increases aqueous humor flow by 19%, timolol decreases flow by 42%, and combined timolol and epinephrine decrease flow by 49%. Intraocular pressures were decreased significantly by epinephrine alone, by timolol alone, and by epinephrine plus timolol.

No additional pressure lowering by epinephrine was seen in timolol-treated eyes in these normal subjects. No difference was seen between any two groups in the endothelial transfer coefficient to fluorescein except between group B (epinephrine) and group D (timolol + epinephrine). The former group was found to have a higher endothelial permeability to fluorescein than the latter group. No differences were found in anterior chamber volume among the four groups.

Discussion. The results of this study indicate that the acute effect of epinephrine on normal eyes pretreated with timolol maleate lowers aqueous humor formation, as exhibited by a decrease in the rate of fluorescein loss from the anterior chamber. This decreased rate of loss could not have come about by a change in outflow resistance, episcleral venous pressure, or uveoscleral outflow. However, we have assumed that the diffusional losses of fluorescein are the same in the timolol-treated eye, with and without epinephrine, namely 10% of the total rate of loss. A 70% change in diffusional

*By paired Wilcoxon test.
Table 1

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Test drug</th>
<th>No. of eyes</th>
<th>Anterior chamber volume ($\mu l$)</th>
<th>Intraocular pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Group 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Timolol</td>
<td>Placebo</td>
<td>23</td>
<td>206.5 ± 31.1</td>
</tr>
<tr>
<td>D</td>
<td>Timolol</td>
<td>Epinephrine</td>
<td>23</td>
<td>206.8 ± 33.7</td>
</tr>
<tr>
<td>Group 2†:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>None</td>
<td>Placebo</td>
<td>20</td>
<td>212.7 ± 20.8</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>Epinephrine</td>
<td>20</td>
<td>212.1 ± 22.1</td>
</tr>
</tbody>
</table>

Values are means ± S.D.
†Data from Townsend and Brubaker (20 of 23 subjects in group 1 were also in group 2).

Table II. Data summary

<table>
<thead>
<tr>
<th>Treatment</th>
<th>p values*</th>
</tr>
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<tbody>
<tr>
<td>Placebo</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>(A)</td>
<td>(B)</td>
</tr>
<tr>
<td>A vs B</td>
<td>B vs C</td>
</tr>
</tbody>
</table>

Aqueous humor flow: $\mu l/min$

- 2.53 ± 0.50 ± 0.68 ± 0.20 ± 0.31 ± 0.01 <0.005 <0.005 <0.05
- 1.47 ± 1.36 ± 0.31 ± 0.01 <0.005 <0.005 <0.05

% increase/decrease from placebo

<table>
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<tr>
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<th>p values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Epinephrine</td>
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<tr>
<td>(A)</td>
<td>(B)</td>
</tr>
<tr>
<td>A vs B</td>
<td>B vs C</td>
</tr>
</tbody>
</table>

Intraocular pressure (mm Hg): Initial

- 12.8 ± 1.94 ± 1.82 ± 0.10 <0.005 <0.005 <0.05
- 8.2 ± 1.75 ± 0.10 <0.005 <0.005 <0.05

Anterior chamber elimination coeff. $(Ke)(min^{-1} \times 10^{10})$

- 7.32 ± 0.18 ± 0.43 ± 0.20 ± 0.70 ± 0.10 <0.005 <0.05 <0.005 <0.05

Endothelial transfer coeff. $(Ka)(min^{-1} \times 10^{10})$

- 4.03 ± 1.43 ± 1.90 ± 0.20 ± 1.23 ± 0.10 <0.005 <0.05 <0.005 <0.05

Values are means ± S.D.
*By paired Wilcoxon test.

losses, if produced by epinephrine, would explain the observed differences in fluorescein loss. We doubt that a change in diffusional loss of this magnitude could have occurred, and thus we assume that part or all of the reduction in fluorescein loss was due to a reduction in aqueous formation.

Tonography was performed on 13 subjects following the fluophotometric measurement. However, the final pressures were so low that interpretation of the tonographic tracing was difficult. Some of the assumptions of tonography are not valid in the hypotonous eye. Thus the tonography results were ignored.

Townsend and Brubaker have shown that the acute effect of epinephrine in the normal eye is to increase aqueous humor production by a mean of 18%. It appears from our data that timolol blocks the stimulatory effects of epinephrine on aqueous humor formation. Coakes and Brubaker, comparing eye to fellow eye, have shown timolol to decrease aqueous formation by 34%. Our data compared to that of Townsend and Brubaker, a comparison of the same eye on two occasions, indicate that timolol decreases aqueous formation by 42%. The combination of epinephrine and timolol appears to decrease aqueous formation by nearly 50% in the normal eye.

It is noteworthy that although the timolol and epinephrine combinations decreased aqueous humor flow, no significant differences appeared in final intraocular pressures between timolol alone and timolol combined with epinephrine. This finding is not surprising, since the rate of aqueous formation is so low that the intraocular pressure...
higher, might yield different results. Patients, in whom intraocular pressure is much hovers near venous pressure. A study of glaucoma disease aqueous formation, and beta agonists seem to decrease aqueous formation slightly, but the alpha effect does not seem to be as important as the beta effect. To further clarify the effects of adrenergic drugs on aqueous formation, a similar experiment using an alpha blocker would be helpful because it is not known whether timolol blocks all the beta effects of epinephrine on the eye or not.

It should be remembered that the acute, single-dose effect of epinephrine on the normal human eye probably differs from the effects of long-term administration. Most published studies in which the effect of epinephrine is measured somewhat later or after long-term administration indicate that aqueous formation is reduced. 2-10 We do not know from this study whether timolol has any influence on the late or long-term effect of epinephrine on aqueous formation. Likewise, it is not known from these studies whether patients with abnormal aqueous humor dynamics respond differently to these drugs than do normals. To know, it will be necessary to measure aqueous humor flow directly in such patients before and after the test drug instillation.

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Key words: aqueous humor, human eye, epinephrine, fluorophotometry, rate of formation, timolol, beta-blocker

REFERENCES


The pathophysiological effects of nitrogen mustard on the rabbit eye. II. The inhibition of the initial hypertensive phase by capsaicin and the apparent role of substance P. C. B. CAMRAS AND L. Z. BITO.

The possibility that the initial, neurogenic ocular hypertensive effect of nitrogen mustard (NM) is mediated by substance P (SP) was investigated by pretreating rabbits with capsaicin, the pungent principle of red pepper, which has been shown to reduce the SP content of primary sensory neurons. One to 3 days after retrobulbar or intracranial pretreatment with capsaicin, no significant changes in intraocular pressure (IOP) or pupillary diameter were observed during the first hour after topical NM application as compared to mean increases in IOP of 3.7 ± 1.9, 9.5 ± 1.3, and 10.8 ± 1.8 mm Hg in "alcohol-denervated" eyes, indomethacin-pretreated animals, and untreated eyes, respectively. Thus the NM-induced initial IOP rise was not affected by indometha-