Reports

Thymoxamine hydrochloride: Effects on the facility of outflow and intraocular pressure. Martin Wand and W. Morton Grant.

Eleven subjects with normal eyes and sixteen subjects with open-angle glaucoma had a selective alpha-adrenergic blocking agent, thymoxamine hydrochloride 0.5 per cent eyedrop, applied to one of their eyes. This consistently produced miosis, but did not significantly alter the intraocular pressure or the tonographically determined facility of aqueous outflow. Because we find that thymoxamine can selectively induce miosis without significant accompanying effect on the ciliary muscle or facility of outflow, we expect that this drug may prove to be a useful diagnostic adjunct to gonioscopy in distinguishing between angle-closure glaucoma and open-angle glaucoma with narrow angle. It seems worthwhile proceeding with clinical evaluation of this possibility.

Thymoxamine hydrochloride is a selective alpha-adrenergic blocking agent, first used in ophthalmology in 1955. This agent has been advocated as a safe and effective miotic for local application after routine dilation of the pupil and for treatment of acute angle-closure glaucoma. We have recognized that there is also potential diagnostic, as well as therapeutic, usefulness for this agent, particularly for distinguishing between angle-closure glaucoma and open-angle glaucoma with narrow angle. To validate the applicability of thymoxamine for this purpose it has been necessary for us first to re-examine the influence of this drug on the facility of outflow and intraocular pressure in normal eyes and in eyes with open-angle glaucoma, because previous reports on these aspects have been confusing and conflicting.

Methods and material. First studied were 11 volunteers with normal intraocular pressures, normal open angles, negative ocular history, average age 35 years (range 20 to 58), including nine males and two females. Baseline applanation tonometry and tonography were performed on each subject. At a later date, the size of the pupil was measured and applanation tonometry was again performed. Then thymoxamine hydrochloride, 0.5 per cent solution, two drops, repeated in two minutes, was put into the conjunctival sac of one eye, either right or left at random. At the end of one hour, the size of both pupils was measured under the same lighting conditions. Applanation tonometry was repeated, and tonography was performed on both eyes. Comparing the treated and untreated eyes, we determined the effects of thymoxamine on the size of the pupil, on intraocular pressure, and on the facility of outflow.

Next studied were 16 patients with open-angle glaucoma, average age 64 years (range 44 to 81), including nine males and seven females. These patients were recently diagnosed as having open-angle glaucoma, but were not yet on any treatment. Each of these patients had a complete ocular examination including gonioscopy, tonography, examination of the optic discs and visual fields to make the diagnosis of open-angle glaucoma, and had no other discoverable ocular abnormality. The effects of thymoxamine on pupil size, intraocular pressure, and the facility of outflow were determined in the same manner as for the normal eyes.

Thymoxamine hydrochloride was supplied in crystalline form by William R. Warner and Company, Limited, England, and was made into the 0.5 per cent eyedrop solution according to their formula by the hospital pharmacy. Official permission (IND 10748) for clinical investigation of a "new drug" was obtained from the United States Food and Drug Administration. Subjects were fully instructed, and they gave their informed consent in writing prior to the use of this agent.

Results. Thymoxamine hydrochloride eyedrops 0.5 per cent consistently produced miosis in normal eyes, with an average miosis of 2.2 mm. (Table I). The average net change in applanation tension was +0.03 mm. Hg, with a range of -1 to +2 mm. Hg, a statistically insignificant change. The average change in the facility of outflow was +0.01, with a range of +0.07 to -0.16. The latter change of -0.16, which was the greatest apparent change, was of low reliability because the baseline tonography curve was of poor quality. The applanation tension in this patient increased only 2 mm. Hg, which suggests that the apparent change in tonography was probably inaccurate. Whether this subject is included or not, the calculated average of change in the facility of outflow is statistically insignificant.

In eyes with open-angle glaucoma, in patients of older average age, miosis was also consistently produced, but the average miosis with only 1.5 mm. (Table II). The average change in applanation tension was -0.02 mm. Hg, with a range of -1 to +1 mm. Hg. The average change in the facility of outflow was 0 with a range of -0.05 to +0.03. These changes in applanation tension and of facility of outflow are statistically insignificant.

None of the patients observed either impair-
Table I. Effect of thymoxamine on normal eyes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Miosis* (mm.)</th>
<th>Change in applanation tension (mm. Hg)</th>
<th>Change in facility of outflow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>+2.0</td>
<td>-0.05</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>+1.0</td>
<td>+0.01</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>0</td>
<td>+0.02</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>-1.0</td>
<td>-0.01</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>0</td>
<td>-0.01</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>0</td>
<td>+0.07</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>0</td>
<td>-0.02</td>
</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>0</td>
<td>+0.02</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>0</td>
<td>-0.04</td>
</tr>
<tr>
<td>10</td>
<td>1.5</td>
<td>+1.0</td>
<td>+0.03</td>
</tr>
<tr>
<td>11</td>
<td>2.0</td>
<td>+2.0</td>
<td>-0.16</td>
</tr>
<tr>
<td>Average</td>
<td>2.2</td>
<td>+0.5</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

*Difference between treated and control pupils.

ment of accommodation or reduction of visual acuity in association with the miosis induced by thymoxamine. None complained of pain during instillation of the drops, but the thymoxamine was always applied shortly after an anesthetic-fluorescein combination had been used for applanation tonometry. Under other circumstances, when a local anesthetic was not used, it was our experience that the thymoxamine eyedrops caused stinging sensation comparable to that produced by most anesthetic eyedrops. The stinging lasted for several minutes. Whether or not anesthetic eyedrops had been used, all eyes showed slight to moderate conjunctival hyperemia within the first few minutes, but by the end of one hour the conjunctiva usually appeared normal. The maximum miotic effect occurred in 30 to 60 minutes, declining during the next few hours. In only one case did miosis last more than 24 hours. No other side effects were observed during this investigation. However, in a related study, one patient developed slight ptosis lasting several hours, and another patient developed epistaxis. In this particular case, the patient has been using a phenylephrine-antihistamine tablet for upper respiratory symptoms. Interestingly, the epistaxis was only on the side where thymoxamine had been used.

Discussion. Although various alpha-adrenergic blocking agents have been used in ophthalmology as early as 1948, all of them had to be administered systemically and all had undesirable side effects. Thymoxamine, which was discovered in 1953, was the first, and to date, the only alpha-adrenergic blocker found to be suitable for topical and systemic use in human beings, inducing negligible local or systemic side effects.

The chemistry and pharmacology of thymoxamine are known in detail. Thymoxamine produces alpha-adrenergic blockade by competitive antagonism. It has no beta-adrenergic blocking effects. Thymoxamine can block the action of phenylephrine, which is an alpha-adrenergic drug, but block only one portion of the actions of epinephrine, which has both alpha- and beta-adrenergic properties. Thymoxamine administered systemically in doses as high as 30 mg. intravenously, or 40 mg. orally per day, or used cutaneously in 10 per cent concentration, has produced only minor side effects, such as facial flushing, vertigo, mild nausea, diarrhea, and headaches.

Published experiences with thymoxamine hydrochloride eyedrops have mentioned transient burning sensation, conjunctival hyperemia, miosis occasionally lasting longer than 24 hours, and ptosis, generally with concentrations greater than 0.5 per cent, but no systemic side effects.

The iris dilator muscle is sympathetically innervated and its receptors alpha-adrenergic in nature. Stimulation produces mydriasis. Blocking by thymoxamine permits the parasympathetically innervated pupillary sphincter to predominate, producing miosis. The ciliary body differs from the iris in that sympathetic stimulation has relatively slight effect on the ciliary muscle, as measured by accommodation, and the ciliary muscle is very largely under parasympathetic control. Because alpha-adrenergic receptors and sympathetic innervational tone have a large role in regulating the size of the pupil, but have very small influence on the ciliary muscle, it is possible with thymoxamine to affect the size of the pupil without affecting the accommodation, and, according to the present findings, to accomplish this without significantly altering the aqueous outflow system.
which is presumably in part under the control of the ciliary muscle.

The dissociation of iris and ciliary muscle responses can not be accomplished satisfactorily by use of cholinergic and anticholinergic drugs, because parasympathetic innervation has a large influence on both iris and ciliary muscle. This is the reason that testing with pilocarpine and related miotics often fails to resolve the sometimes difficult clinical dilemma of distinguishing between true angle-closure glaucoma and open-angle glaucoma associated with an angle so extremely narrow that one cannot be sure gonioscopically whether it is open or closed. Pilocarpine can reduce the pressure and improve the facility of outflow by two mechanisms (at least), by opening the angle or by acting on the outflow system itself through the ciliary muscle. This can leave interpretation of the result uncertain, yet a decision whether to treat medically or surgically often rests upon resolving this differential diagnosis.

The clinical studies that we have described have shown that thymoxamine can cause the pupil to constrict without affecting the intraocular pressure or the facility of outflow in normal eyes and in eyes with open-angle glaucoma. One previous study reported that 0.5 per cent thymoxamine eyedrops reduced the intraocular pressure in 15 normal subjects, but the documentation seems to have been inadequate. Another study is reported to have found that 1 per cent thymoxamine had no effect on the intraocular pressure in seven patients with open-angle glaucoma, but that the facility of outflow was reduced one week after continued use of thymoxamine. The controls and documentation in this study also seem inadequate.

From our results in normal and open-angle glaucomatous eyes, which we wish to regard as controls for further studies, we postulate that if a glaucomatous eye with an apparently closed angle is treated with thymoxamine 0.5 per cent eyedrops and both the intraocular pressure and the facility of outflow are improved commensurate with a gonioscopic appearance of conversion from closed to open angle, one should be able to conclude that this confirms a diagnosis of angle-closure glaucoma. If, instead, thymoxamine testing induces miosis with gonioscopically evident opening of the angle, but does not normalize the pressure and facility of outflow, it seems clear that one should diagnose open-angle glaucoma as the fundamental problem. Validation of this concept will of course require further clinical examination in patients who present this particular dilemma, with the final assessment to be based on analysis of the results of treatment.

From the Glaucoma Consultation Service, Massachusetts Eye and Ear Infirmary, and the Howe Laboratory of Ophthalmology, Harvard Medical School, Boston. This study was supported by United States Public Health Service Research Grant 2-ROI-EY00002 from the National Eye Institute. Dr. Wand was supported by a Heed Ophthalmic Foundation Fellowship. Submitted for publication Nov. 17, 1975. Reprint requests: Dr. M. Wand, 100 Retreat Ave., Hartford, Conn. 06106.

Key words: thymoxamine hydrochloride, alpha-adrenergic blockers, facility of outflow, intraocular pressure, angle-closure glaucoma, open-angle glaucoma with narrow angles.

REFERENCES
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Differential sensitivity at the cellular level
in primary open-angle glaucoma: prednisolone and ouabain. PAUL F. PALMBERG, DAN RACHLIN, AND BERNARD BECKER.

The concentration of drug necessary to inhibit lymphocyte transformation by 50 per cent (I50 value) was determined for the glucocorticoid prednisolone-21-phosphate, and for the sodium-potassium adenosine triphosphatase inhibitor ouabain, in 37 persons representing the full range of ocular and cellular glucocorticoid sensitivity. The lymphocytes of patients with primary open-angle glaucoma, and of high ocular responders to glucocorticoids, were more sensitive to prednisolone-21-phosphate than were those of ocular low and intermediate responders. By contrast, there was no significant difference in sensitivity to ouabain. There was also no significant correlation of lymphocyte sensitivity to prednisolone-21-phosphate with that to ouabain. These results provide indirect evidence that the increased cellular sensitivity to glucocorticoids in primary open-angle glaucoma is a specific effect, and not merely representative of a general vulnerability of "sick cells."

Persons with primary open-angle glaucoma have been shown to have increased sensitivity to glucocorticoids in a variety of tissues, including the eye,1-14 hypothalamic-pituitary gland,15-16 skin fibroblasts,17 and peripheral blood lymphocytes.8,8 This increased sensitivity to glucocorticoids may be relevant to the etiology of the disease since in the eye topically applied glucocorticoids have been found to elevate intraocular pressure and mimic glaucoma.4,10-11 Furthermore, primary open-angle glaucoma patients as a group showed particularly high ocular responses to topical glucocorticoids, as in a standardized six-week test 82 per cent reached intraocular pressures of > 31 mm Hg, while only 6 per cent of persons in the general population did so.3

The lymphocytes of patients with primary open-angle glaucoma, and of high ocular responders to glucocorticoids, have been found to be more sensitive than those of normal persons to both the glucocorticoid prednisolone-21-phosphate, and the cyclic adenosine monophosphate (c-AMP) phosphodiesterase inhibitor theophylline.12 The finding of increased sensitivity to two distinct classes of drugs raised the question whether that increased sensitivity to these drugs was specific, or whether it represented a general vulnerability of "sick cells." In order to answer that question more directly the underlying molecular mechanisms had to be investigated. To that end studies of cytosol glucocorticoid receptor concentrations and affinities have been undertaken. In addition, theophylline inhibition of c-AMP phosphodiesterase was compared in lymphocytes from glaucoma patients and normal subjects.13 The latter study failed to demonstrate differences in phosphodiesterase affinity for theophylline, or in levels or metabolism of c-AMP in lymphocytes from glaucoma patients and normal subjects. Studies of the actions of c-AMP on subsequent metabolic steps have been started.

While attempts to find a specific molecular lesion have continued, other more indirect approaches have been explored. In the present study we have determined the sensitivity of lymphocytes to a third type of drug, the Na+-K+-ATPase inhibitor ouabain14 and compared this with prednisolone-21-phosphate using parallel assays.

Materials and methods. The 37 persons studied included four previously classified as ocular low responders, one intermediate responder, seven high responders,2 two patients with primary open-angle glaucoma, and 23 persons not topically classified. The unclassified persons included two patients with chronic-angle closure glaucoma, 13 with pigmented glaucoma, two with low-tension glaucoma, two with inactive glaucomatocyclitic crisis, and four normal subjects. The five low and intermediate responders were 51 ± 12 (σ) years old, and the nine primary open-angle glaucoma patients and ocular high responders were 63 ± 12 years old.

Ouabain was obtained from the Sigma Chemical Company, St. Louis. Prednisolone-21-phosphate was a gift from Merck, Sharp, and Dohme, West Point, Pa.

Peripheral blood samples from fasted patients
Table I. \(I_0\) values for inhibition of lymphocyte transformation by prednisolone-21-phosphate and ouabain for 14 patients classified by their ocular response or the presence of primary open-angle glaucoma

<table>
<thead>
<tr>
<th>Ocular classification</th>
<th>No.</th>
<th>Prednisolone-21-phosphate (I_0)</th>
<th>Ouabain (I_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular low or intermediate response</td>
<td>5</td>
<td>150 ± 49 nM</td>
<td>28 ± 6 nM</td>
</tr>
<tr>
<td>Ocular high response or open-angle glaucoma</td>
<td>9</td>
<td>82 ± 27 nM</td>
<td>31 ± 5 nM</td>
</tr>
</tbody>
</table>

*For prednisolone-21-phosphate the differences in \(I_0\) values are significant (\(p < 0.005\)).

Table II. \(I_0\) values for inhibition of lymphocyte transformation by prednisolone-21-phosphate and ouabain, for 37 patients classified by in vitro response

<table>
<thead>
<tr>
<th>In vitro glucocorticoid sensitivity category</th>
<th>No.</th>
<th>Prednisolone-21-phosphate (I_0)</th>
<th>Ouabain (I_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&gt;146 nM)</td>
<td>14</td>
<td>182 ± 25 nM</td>
<td>29 ± 6 nM</td>
</tr>
<tr>
<td>Intermediate (105-146 nM)</td>
<td>11</td>
<td>118 ± 8 nM</td>
<td>29 ± 6 nM</td>
</tr>
<tr>
<td>High (&lt;105 nM)</td>
<td>12</td>
<td>66 ± 16 nM</td>
<td>28 ± 6 nM</td>
</tr>
</tbody>
</table>

were collected in heparinized syringes between 8 and 9 A.M. on the day of assay. Details of cell preparation, pre-incubation of cells with prednisolone-21-phosphate, subsequent mitogen addition, radiolabeled thymidine addition, cell harvesting, scintillation counting, and computation of \(I_0\) values have been reported previously.\(^8-9\) Assays with ouabain were performed in exactly the same manner except that ouabain was substituted for prednisolone-21-phosphate. The concentrations of ouabain used were 1.78-, 2.37-, 3.16-, and 4.22 \(\times 10^{-8}\) M.

Results.

Responses considered with reference to ocular classification. In agreement with previous findings,\(^6-9\) the nine persons who were either ocular high responders or primary open-angle glaucoma patients had significantly greater sensitivity to prednisolone-21-phosphate than did the five who were either low or intermediate ocular responders (Table I). There was, however, no significant difference in sensitivity to ouabain between the two groups.

Responses considered with reference to in vitro glucocorticoid sensitivity. The 37 persons were also classified as low, intermediate, or high responders to prednisolone-21-phosphate in vitro, using categories corresponding to the three ocular response groups.\(^13\) When this was done, 14 (aged 51 ± 16 years) were classified as in vitro low responders, 11 (aged 46 ± 20) as intermediate, and 12 (aged 54 ± 14) as high responders. There was no significant difference in sensitivity to ouabain among the three groups (Table II).

The data were also considered without reference to in vitro categories by performing regression analysis for the ouabain and prednisolone-21-phosphate \(I_0\) values. No significant correlation (\(r = 0.032\)) was found.

Neither the \(I_0\) values for prednisolone-21-phosphate (\(r = 0.059\)), nor those for ouabain (\(r = 0.187\)) correlated significantly with patient age.

Discussion. The in vitro responses to prednisolone-21-phosphate of the 14 persons who were previously classified by ocular response or were known to have primary open-angle glaucoma are consistent with previous findings. As before, the lymphocytes of patients with primary open-angle glaucoma, and of ocular glucocorticoid high responders, are more sensitive to glucocorticoids than are those of ocular low and intermediate responders.\(^9\) The molecular basis for this increased sensitivity, and the role it may play in the etiology of glaucoma, remain unknown.

The concentration of ouabain found to reduce lymphocyte transformation by one-half ranged from 1.9- to 4.0 \(\times 10^{-8}\) M, and averaged 2.9 \(\times 10^{-8}\) M for the 37 subjects. The \(K_i\) for ouabain inhibition of Na+-K+-ATPase is reported to vary greatly from tissue to tissue and species to species, but usually is from \(10^{-7}\) to \(10^{-9}\) M.\(^14\) As the \(K_i\) for inhibition of lymphocyte Na+-K+-ATPase was not measured directly in the present study, we may only assume that ouabain influenced transformation by inhibiting this enzyme.

We find no correlation between increased ocular or cellular sensitivity to glucocorticoids and sensitivity to ouabain. This is in marked contrast to the previous results for prednisolone-21-phosphate and theophylline, in which a significant correlation of sensitivities was found (\(r = 0.748\)).

Since the lymphocytes of in vitro glucocorticoid high responders are not more sensitive to ouabain than those of low responders, they do not appear to be merely more vulnerable or nonspecifically "sick cells." The results provide some indirect evidence for the specificity of the increased responses to prednisolone-21-phosphate and theophylline. Direct evidence, however, must come from further studies of the underlying molecular mechanisms.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, Mo. This study was supported in part by Grants...
Effect of long-wave ultraviolet light on the lens. I. Model systems for detecting and measuring effect on the lens in vitro.

JOHN F. R. KUCK, JR.

Rat, mouse, and chick lenses incubated with 3-aminotriazole under long-wave ultraviolet (UV) show reduced accumulation and incorporation of leucine and a loss of glutathione. The effect on leucine incorporation is strikingly enhanced when capsule-epithelium pools are incubated. The procedure may identify photosensitizers or metabolic inhibitors which are cataractogenic when acting in conjunction with UV.

The UV normally reaching the lens is at 293 to 420 nm; thus a consideration of cataractogenesis must be restricted to this range. In addition the effect of photosensitizers or metabolic inhibitors may be considerable, giving rise to the type of cataractogenesis discussed by Hockwin and Koch. The minimal effective UV as observed by Bachem is increased in the presence of a photosensitizer. Clinical evidence suggests possible involvement of UV in human senile cortical cataract. Recently, in vivo mouse irradiation experiments have given firm evidence for the cataractogenic potential of UV.

This report concerns experiments designed to give a rapid response by lenses exposed to UV while incubating in medium containing the catalase inhibitor, 3-aminotriazole (AT). Damage to the lens is measured by a fall in amino acid accumulation or incorporation. By using an unnaturally high intensity of UV, an effect is achieved which could come from sunlight only after years of exposure. The enhancement of this damaging effect by an agent such as AT may indicate the nature of substances in the environment which greatly potentiate the normally low cataractogenic capability of UV.

Materials and methods. The UV source was a "black-light" lamp immersed in the incubation bath so that lenses in transparent vessels were about 2 to 3 mm. from the lamp surface at a flux intensity of about 2.5 mwatts per square centimeter. In addition an overhead lamp added another 1.5 mwatts per square centimeter. The lenses were incubated at 37° C. in a glucose-balanaced salt solution based on carbonate-bicarbonate gassed with 95 per cent O₂ - 5 per cent CO₂ as the buffer. Circulation of medium and bath water was maximized to maintain the same temperature in UV-exposed and shielded tubes. The medium above capsule-epithelium pools was gently circulated so that they lay in proximity to the lamp without moving. The medium contained AT at 5 or 10 mM and the added tracer amino acid. Amino acid accumulation or incorporation and a loss of glutathione. The effect on leucine incorporation is strikingly enhanced when capsule-epithelium pools are incubated. The procedure may identify photosensitizers or metabolic inhibitors which are cataractogenic when acting in conjunction with UV.