time to the enzyme was 24 hours at a concentration of 8,200 units per milliliter which would provide both an adequate contact time and enzyme concentration for glycosaminoglycan degradation. The quantitative examination of stromal tissue sections for glycosaminoglycans revealed that hyaluronic acid was lost entirely from the stroma and other sulfated compounds were also lost from the tissue. The relationship between hyaluronidase degradation and ion binding has been adequately demonstrated in the arterial wall, and a similar situation appears to exist in the stroma; that is that the capability for sodium binding by glycosaminoglycans is markedly reduced.

The free acid sites in the stroma are one of the major contributing factors to the swelling pressure of the stroma, and direct measurements reveal that this Donnan (or electrostatic) pressure is about one-half of the total swelling pressure of rabbit stroma in physiological solution and at near-normal hydration. After digestion with hyaluronidase the swelling capacity of the stroma is markedly inhibited, thus indicating that a major factor normally causing swelling has been negated. Enzymatic digestion with testicular hyaluronidase, therefore, appears to remove glycosaminoglycans from the corneal stroma very effectively and offers a means of studying the behavior of corneal stroma which is free of acidic sites generated by the glycosaminoglycans.

If the depolymerization of glycosaminoglycans is complete, and the quantitative histochemical data indicates a loss of about 85 to 90 per cent of these compounds, the reduction of the bound sodium value to one-half that of a normal stroma indicates that about one-half of the total bound sodium is located on anionic sites elsewhere in the stroma. The reduction in net fixed charge and the reduction in total bound sodium, both found here, provides direct experimental verification of the previous data analysis based on the results from a large number of stromas under various ambient bathing conditions and at different hydrations, which revealed that approximately one-half of the total stromal bound sodium is on nonsaccharide sites.

We thank Dr. T. H. Rosenquist, of the Department of Anatomy, for performing the tissue mucopolysaccharide analyses and Ms. Debbie Hancock for her secretarial help. From the Departments of Ophthalmology and Physiology, Medical College of Georgia, Augusta, Ga. 30902, and the W. K. Kellogg Research Laboratories, The Wilmer Institute, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205. Supported in part by United States Public Health Service Research Grants EY 00034 and EY 01413. Dr. Green was the recipient of a Public Health Service Research Career Development Award 1 K4 EY 46 354 from the National Eye Institute during part of this work. Submitted for publication Feb. 24, 1975. Reprint requests: Dr. Keith Green, Department of Ophthalmology, 3 D 11, R & E Building, Medical College of Georgia, Augusta, Ga. 30902.

Key words: ion binding, rabbit, corneal stroma, glycosaminoglycans, hyaluronidase degradation, sodium binding sites.

REFERENCES


Pathways of the eye's response to topical nitrogen mustard. Lee M. Jampol, Alan Axelrod, and Howard Tessler.

We studied the effect of prior corneal herpes simplex infection with its resultant corneal hypesthesia on the irritative response of the rabbit eye to topical nitrogen mustard. Both the miosis and the breakdown of the blood-aqueous barrier that follow the application of topical nitrogen mustard were diminished in eyes infected three weeks previously with herpes simplex virus. Nonspecific corneal scarring did not affect the response. This suggests again that an axon reflex requiring intact sensory innervation mediates the
response to nitrogen mustard. Pretreatment of normal (noninfected) rabbits with systemic H, and H, antihistamines, topical scopolamine hydrobromide, or topical and systemic corticosteroids was ineffective in blocking the miosis or increased protein in the aqueous humor following topical nitrogen mustard.

Application of topical 1 per cent nitrogen mustard to the rabbit eye results in an irritative response characterized by conjunctival and iridal hyperemia, ocular hypertension, increased protein in the aqueous humor, and miosis. Aspirin, an inhibitor of prostaglandin synthesis, does not block the protein rise following nitrogen mustard, and following topical nitrogen mustard, prostaglandins cannot be consistently demonstrated in the aqueous humor (K. Eakins, unpublished data). Denervation of the eye with retrobulbar alcohol attenuates the miosis and the protein rise in the aqueous humor following topical nitrogen mustard. Thus, the response to nitrogen mustard is apparently not mediated by prostaglandins but is dependent on intact innervation. The present work describes further studies on the response to nitrogen mustard. The first part investigates the effect of prior ocular herpes simplex infection on the response to nitrogen mustard. The second part describes the effect of pretreatment of noninfected rabbits with antihistamines, parasympatholytic agents, or corticosteroids on the response to nitrogen mustard.

Methods and materials. All experiments utilized 2.5 to 4 kilogram male New Zealand albino rabbits, lightly anesthetized with sodium pentobarbital. Two drops of 1 per cent nitrogen mustard (mechlorethamine hydrochloride) were applied to both corneas of each rabbit. After 30 minutes pupillary diameters were measured with a hand-held pupil gauge. Anterior chamber paracentesis was performed and the aqueous humor from each eye was assayed for protein by the method of Lowry and co-workers. Preliminary experiments demonstrated that no consensual irritative response was present in the contralateral eye 30 minutes after the application of 1 per cent nitrogen mustard to one eye of a rabbit; each eye of a given rabbit was thus considered a separate experiment. All results in this paper are expressed as mean ± S.E.M. (n). Duncan’s new multiple range test was used to determine statistical significance.

The effect of prior herpes simplex infection. Herpes simplex keratouveitis was produced in both eyes of six rabbits by abrasion of the corneal epithelium with a 19-gauge needle and the application of two drops of PH strain Herpesvirus hominis-type one to the cul-de-sac. Over the next three days keratouveitis developed, characterized by corneal epithelial and stromal involvement and moderate uveitis. Three weeks after the viral inoculation, the rabbits showed corneal scarring with vascularization, corneal hypesthesia (ranging from mild to marked), normally reactive pupils with no synechiae, and no remaining aqueous flare or cells. After the induction of light anesthesia, 1 per cent nitrogen mustard was applied to both eyes of these rabbits and the pupillary diameters and the protein levels in the aqueous humor were determined 30 minutes later.

Three rabbits had both their corneas abraded with a 19-gauge needle without viral inoculation. This resulted in a milder keratitis and uveitis that resolved over three days. Three weeks later these animals showed only mild corneal scarring and normal corneal sensation. One per cent nitrogen mustard was applied to both eyes of these rabbits, and the pupillary diameters and protein levels in the aqueous humor were determined as above.

The effects of antihistamines, scopolamine, and corticosteroids. Antihistamines. One hundred milligrams of promethazine hydrochloride, an H, antihistamine, was given intramuscularly to three anesthetized rabbits. Within 15 minutes, both pupils of each rabbit became fixed and dilated. Thirty minutes after the promethazine, 1 per cent nitrogen mustard was applied to both eyes of these rabbits, and the protein level in the aqueous humor and pupillary diameters were measured 30 minutes later.

One hundred twenty two milligrams of metiamide, a newly synthesized H, antihistamine was given intramuscularly to six rabbits and intraperitoneally to four additional rabbits. One hour later 1 per cent nitrogen mustard was applied to both eyes of all rabbits, and the aqueous humor and pupillary diameters were determined 30 minutes later.

Topical parasympatholytic agents. Two drops of 0.25 per cent scopolamine hydrobromide (Isopto Hyoscine) were applied topically to both eyes of seven rabbits 60 minutes and 30 minutes prior to the application of nitrogen mustard. Protein levels in the aqueous humor and pupillary diameters were determined 30 minutes after topical nitrogen mustard was applied to both eyes of each rabbit.

Corticosteroids. Three rabbits received one drop of 1 per cent prednisolone sodium phosphate topically in each eye 60, 45, and 30 minutes prior to topical application of the nitrogen mustard, and 75 mg. of hydrocortisone sodium succinate intravenously 30 minutes before nitrogen mustard application. Protein levels in the aqueous humor and pupillary diameters were measured 30 minutes after nitrogen mustard application.

Results. Following application of 1 per cent topical nitrogen mustard to the rabbit eye, the miosis, aqueous protein elevation, and ocular hypertension all reached maximal levels within
Table I. Protein levels in the aqueous humor 30 minutes after topical 1 per cent nitrogen mustard.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Protein* (mg. per cent)</th>
<th>Significancef</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (normal eye)</td>
<td>1,660 ± 156 (22)</td>
<td>—</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>816 ± 166 (12)</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Corneal scarring</td>
<td>1,480 ± 192 (6)</td>
<td>NS‡</td>
</tr>
<tr>
<td>Promethazine HCl</td>
<td>1,476 ± 145 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Metiamide (intramuscular)</td>
<td>1,740 ± 142 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Metiamide (intraperitoneal)</td>
<td>2,208 ± 235 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>1,266 ± 130 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>1,249 ± 287 (6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M. (n).
†Compared to normal eyes.
‡NS—not significant at the 5 per cent level.

20 to 30 minutes. Thirty minutes after the application of nitrogen mustard 22 normal rabbit eyes showed an increase in aqueous humor protein from normal values of about 50 mg. per cent to 1,660 ± 156 mg. per cent (Table I). Intense miosis developed in most eyes and the pupillary diameter at this time was usually 2 to 3 mm.

**Herpes simplex infection.** Thirty minutes after the topical application of nitrogen mustard the 12 eyes previously infected with herpes simplex showed a rise in aqueous humor protein to only 816 ± 166 mg. per cent (Table I). This reaction was significantly less than that shown in normal eyes (P < 0.01). In many eyes there was also a partial inhibition of the miosis.

Nonspecific corneal scarring without herpetic inoculation did not influence the response to topical nitrogen mustard. Thirty minutes after nitrogen mustard, six eyes in this category showed a rise in protein to 1,480 ± 192 mg. per cent, not significantly different from normal eyes (Table I). The miosis was also unaffected.

**Antihistamine, scopolamine, and corticosteroid effects.** Pretreatment with intramuscular promethazine hydrochloride, intramuscular and intraperitoneal metiamide, topical scopolamine hydrobromide, or systemic and topical corticosteroids were all ineffective in preventing either the protein rise or miosis after topical nitrogen mustard instillation (Table I).

**Discussion.** Although the irritative response of the rabbit eye to paracentesis, alkali burns, and iris trauma appears to be mediated in part by prostaglandins, this is not true of all irritative stimuli. Topical nitrogen mustard, intracameral formaldehyde, and antidromic stimulation of the fifth nerve elicit miosis, hyperemia, ocular hypertension, and increased protein in the aqueous humor; however, these effects do not appear to be mediated by prostaglandins. Our previous work has shown that interruption of the innervation of the eye with retrobulbar alcohol can prevent the breakdown of the blood-aqueous barrier and miosis that follows application of topical nitrogen mustard. The similarity of the response to nitrogen mustard to antidromic fifth nerve stimulation, and its dependence on innervation make it tempting to speculate that the response is an axon reflex, mediated in both the afferent and efferent arcs by pain (sensory) fibers. The humoral mediator of this response is apparently not a prostaglandin.

Prior herpes simplex infection was another means to assess the role of sensory innervation in the response to topical nitrogen mustard. Herpes keratitis produces corneal hypesthesia, and histologic studies in the rabbit have demonstrated destruction of stromal corneal nerves. Prior herpetic infection did attenuate the miosis and the increase in aqueous protein levels following topical nitrogen mustard (Table I). Nonspecific corneal scarring did not affect the corneal sensation or the response to nitrogen mustard. These data support the hypothesis that the nitrogen mustard response does require intact sensory innervation. However, since it is well known that herpetic infection can cause damage to many intraocular structures including the autonomic innervation, the iris, and the ciliary body, it is not certain that the attenuation of the response to nitrogen mustard was due only to destruction of the sensory innervation.

The classic H1 antihistamine promethazine hydrochloride did not prevent the miosis or the protein rise in the aqueous humor following topical nitrogen mustard. Antihistamines have previously been found ineffective in preventing the appearance of protein in the aqueous humor following fifth nerve section in the rat. A second histamine receptor (the H2 site) is present in the gastric mucosa and cardiac atrium, and is not blocked by classic (H1) antihistamines. Systemic administration of metiamide, an H2 antihistamine was also ineffective in preventing the ocular response to nitrogen mustard. However, the ocular penetration of this new drug is uncertain and this may account for its lack of effect.

Topical scopolamine hydrobromide or the combination of topical and systemic steroids also did not effect the miosis or the increase in protein in the aqueous humor following nitrogen mustard.

On the basis of our present experiments, we feel that it is unlikely that the miosis and the increase in aqueous protein that follow topical nitrogen mustard are mediated solely by histamine or acetylcholine. We have not, however, completely ruled out such a role. Previous studies have suggested that prostaglandins are not involved and that the sympathetic innervation is unimportant.

Further work on the possible role of the kinins, serotonin, and other inflammatory mediators is
necessary to elucidate the humoral mediator of this response.

We thank Edward Cotlier, M.D. for the use of his laboratory. Jang Oh, M.D. supplied the herpes simplex virus. John W. Paul, Smith, Kline, and French Laboratories kindly supplied the metamide. Minu Patel, M.S. assisted with the statistical evaluation of the data.

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Key words: nitrogen mustard, innervation, axon reflex, irritative response, herpes simplex, antihistamine, corticosteroid, scopolamine, prostaglandin.

REFERENCES


Timolol ophthalmic solutions 0.5 per cent, 1.0 per cent, and 1.5 per cent lowered intraocular pressures significantly in normal human volunteers. Maximum lowering of the intraocular pressures was reached at two hours with the 0.5 per cent solution of timolol and at one hour with the 1.0 per cent and 1.5 per cent timolol ophthalmic solutions. The effect lasted the full seven hours of observations. No objective or subjective evidence of ocular irritation could be attributed to the drug. A single dose of timolol applied topically to the eyes of normal human volunteers had no effect on pupillary size, visual acuity, blood pressure, or pulse rate.

Since the introduction of the carbonic anhydrase inhibitors and the reintroduction of epinephrine some 20 to 25 years ago, no significant new drug therapy has been introduced in the therapy of chronic open-angle glaucoma.

There is a definite medical need for a new effective antiglaucoma drug. If an effective new drug can be administered topically to the eye without creating the annoying side effects observed with the readily available cholinergic or adrenergic agents, a significant breakthrough in the treatment of chronic open-angle glaucoma would result. With present drug therapy, the decision to treat frequently does not depend on the degree of pressure abnormality and the effectiveness of the medication, but on consideration of the adverse effects from therapy.

Timolol, a beta-adrenergic blocking agent, may be such a drug. It lacks intrinsic sympathomimetic activity. It also lacks the local anesthetic activity found with propranolol. Milligram for milligram, timolol is about eight times more potent than propranolol when administered systemically. Extensive experience with the administration of timolol orally and intravenously has demonstrated safety of the compound in humans.

Timolol and a number of reference agents have been studied with respect to their ability to lower intraocular pressure (IOP) of rabbits with experimental glaucoma induced by intraocular injection of α-chymotrypsin. These studies demonstrate that 0.5 per cent and 1.5 per cent concentrations of timolol are very effective in lowering IOP after topical application. Pressure was reduced in the normal and the glaucomatous rabbit eye, although the latter responded to a greater extent than the normal. The effect of timolol in lowering IOP compared favorably with standard antiglaucoma agents such as topical epinephrine.