Pathways for the response of the eye to injury

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The role of a neural pathway in the disruption of the blood-aqueous barrier of the rabbit after two irritating stimuli, topical nitrogen mustard, and paracentesis, was studied. Retrobulbar anesthesia or section of the ophthalmic division of the trigeminal nerve decreased the breakdown of the blood-aqueous barrier, as measured by protein in the aqueous humor, after topical nitrogen mustard. Sensory denervation, accomplished with retrobulbar alcohol, virtually prevented the protein rise in the aqueous humor. Disruption of the blood-aqueous barrier after paracentesis, however, was not affected by retrobulbar anesthesia or alcohol denervation. Therefore, the increased protein in the aqueous humor after an irritative stimulus appears to be mediated by at least two pathways. The response to a stimulus such as nitrogen mustard depends on sensory innervation and is not mediated by prostaglandins to any important degree. The response to paracentesis does not require sensory innervation and appears to be mediated, at least in part, by prostaglandins.

Key words: nitrogen mustard, paracentesis, innervation, prostaglandin, irritative response, blood-aqueous barrier.

The eye immediately responds to irritants with conjunctival and iridial hyperemia, increased protein in the aqueous humor, increased intraocular pressure, and miosis. The response is seen clinically after conjunctivitis or corneal abrasion and other trauma, and has often been described under experimental conditions in the rabbit eye. In the latter instances it has been shown that local anesthesia or trigeminal section will attenuate the ocular hyperemia; that there is both a mechanical and neural component to the iridial hyperemia; and that the neural component has an extrabulbar pathway. In the latter study an attempt was made to separate the four components of the irritative response as well as to delineate the requirement for an extrabulbar pathway. The present work continues these studies, incorporating recent information about the prostaglandins as possible mediators of the irritative response, and, in particular, evaluating the means by which one component, increased aqueous
humor protein, develops after the irritants, topical nitrogen mustard, and paracentesis.

Materials and methods

All experiments utilized male New Zealand albino rabbits, 2.5 to 4 kilograms, lightly anesthetized with sodium pentobarbital. Protein levels in the aqueous humor were determined by paracentesis of the anterior chamber and assay of the fluid for protein using the method of Lowry and co-workers. All results are expressed as mean ± S.E.M. (n). Statistical significance was determined using Student's t-test.

Nitrogen mustard.

Retrobulbar lidocaine. Eleven rabbits were lightly anesthetized and given 0.7 ml of retrobulbar 2 per cent lidocaine with 50 units of hyaluronidase to one eye. A control injection of 0.7 ml of normal saline with hyaluronidase was given to the other eye. Five minutes later, two drops of 1 per cent nitrogen mustard (mechlorethamine hydrochloride) were placed in the cul-de-sac of both eyes. Twenty minutes later, protein levels in the aqueous humor were determined.

Seven rabbits were given retrobulbar lidocaine and saline as above. Five minutes later two drops of 0.2 per cent prostaglandin E (PGEJ) were applied to both eyes. Twenty minutes later, protein in the aqueous humor was measured.

Preliminary experiments done with topical anesthesia showed only incomplete blockade and therefore retrobulbar delivery of anesthesia was exclusively employed. Other preliminary experiments showed that 0.2 per cent nitrogen mustard and 1 per cent nitrogen mustard gave similar results. Using the latter dose ensured a uniformity of response.

Superior cervical ganglionectomy. Unilateral superior cervical ganglionectomy was performed to develop postganglionic sympathetic denervation. Three weeks later, nitrogen mustard was applied to both operated and control sides. Protein levels in the aqueous humor were measured 30 minutes later.

Section of the ophthalmic division of the fifth nerve. Unilateral intracranial section of the ophthalmic division of the fifth nerve was performed on seven rabbits. Under the operating microscope, a portion of the skull was removed between the orbit and ear, exposing the temporal parietal cortex. After removing some brain tissue, the fifth nerve was seen exiting from the Canserian ganglion and the ophthalmic branch was sectioned postganglionically. Three weeks later, nitrogen mustard was applied topically to the operated and control sides and protein in the aqueous humor was determined thirty minutes later.

Retrobulbar alcohol. A unilateral retrobulbar injection of 8.0 ml of absolute ethanol was administered to seven rabbits. This resulted in considerable orbital and some ocular inflammation which resolved over one to two weeks. Topical and systemic antibiotics were administered for several days. Any animal developing significant corneal ulceration or uveitis was discarded. Two weeks later, nitrogen mustard was given to the alcohol-treated and control eyes and the protein in the aqueous humor measured at 30 minutes.

Paracentesis.

Retrobulbar lidocaine. Six rabbits were used for retrobulbar lidocaine and saline injections, as described above. Five minutes later, paracentesis of the anterior chamber was performed. Fifteen minutes later, protein in the aqueous humor was determined.

Retrobulbar alcohol. Six rabbits were given unilateral retrobulbar alcohol as described above. Two weeks later paracentesis was performed bilaterally. Protein in the aqueous humor was determined 30 minutes after paracentesis.

Retrobulbar alcohol and aspirin. Another series of experiments utilized five rabbits that two weeks before had received unilateral retrobulbar alcohol. One hour prior to paracentesis these animals were pretreated with 600 mg. of aspirin per rectum to prevent prostaglandin synthesis. Thirty minutes after paracentesis, protein levels in the aqueous humor were measured.

Results

Nitrogen mustard.

Retrobulbar lidocaine. Twenty minutes after topical nitrogen mustard (Fig. 1),
Fig. 2. Effect of trigeminal section and alcohol denervation on the breakdown of the blood-aqueous barrier after nitrogen mustard. Protein in the aqueous humor was determined thirty minutes after nitrogen mustard. The two left bars compare the protein in the aqueous humor of control eyes and eyes three weeks after ophthalmic nerve section (Op.). The difference is not statistically significant (0.05 < p < 0.10). The two right bars compare the protein in the aqueous humor of control and alcohol-denervated eyes (Inj.). The difference is significant (p < 0.01).

eyes that were pretreated with retrobulbar saline showed a rise in the protein in the aqueous humor to 1,167 ± 200 mg per cent (11). The opposite eye, previously pretreated with retrobulbar lidocaine showed a rise to 500 ± 75 mg per cent (11). Thus, retrobulbar lidocaine resulted in greater than 50 per cent inhibition of the aqueous protein response to nitrogen mustard. There was, however, little effect on the miosis and iris ischemia that follow topical nitrogen mustard.

The lidocaine inhibition of the protein response to nitrogen mustard was not due to a change in sensitivity to prostaglandins because retrobulbar lidocaine did not influence the increase in protein in the aqueous humor in response to topical PGE₂. Twenty minutes after 0.2 per cent topical PGE₂, saline-injected eyes showed a protein in the aqueous humor level of 907 ± 130 mg per cent (7). Lidocaine-injected eyes showed a similar rise in protein to 779 ± 108 mg per cent (7).

Superior cervical ganglionectomy. Ganglionectomy did not alter the response to topical nitrogen mustard. Thirty minutes after nitrogen mustard, the sympathectomized eyes showed a rise in the protein in the aqueous humor to 1,447 ± 219 mg per cent (7). The control eyes of the same animals rose to 1,412 ± 191 mg per cent (10). Thus, the partial prevention of the response to nitrogen mustard by retrobulbar lidocaine is not mediated through blockade of the sympathetic nervous system.

Trigeminal section. Unilateral section of the ophthalmic division of the fifth nerve resulted in a marked decrease in ipsilateral corneal sensation. A somewhat decreased breakdown of the blood-aqueous barrier, after topical nitrogen mustard occurred on the operated side (Fig. 2). The protein in the aqueous humor of control eyes rose to 2,020 ± 255 mg per cent (7); the protein in the aqueous humor of eyes on the operated side rose to 1,330 ± 266 mg per cent (7). However, the difference was not statistically significant (0.05 < p < 0.10). The corneal reflex in these animals was not totally absent and we think that some persistent innervation, unaffected by surgery, mediated the residual response to nitrogen mustard.

Retrobulbar alcohol. Pretreatment with retrobulbar alcohol abolished the corneal reflex and almost completely prevented the responses of the rabbit eye to topical nitrogen mustard. There was marked inhibition of the miosis, iris ischemia, and increased protein in the aqueous humor (Fig. 2). Thirty minutes after topical nitrogen mustard, control eyes showed a rise in protein in the aqueous humor to 1,726 ± 367 mg per cent (6). The aqueous humor of eyes pretreated with retrobulbar alcohol rose only to 163 ± 78 mg per cent (7).

Paracentesis. Pretreatment with retrobulbar lidocaine or denervation with alcohol did not affect the rise in protein in the aqueous humor after paracentesis (Fig.
3). Fifteen minutes after paracentesis, the protein in the aqueous humor of eyes treated with retrobulbar saline and lidocaine was $3,978 \pm 238$ mg. per cent (6) and $3,951 \pm 385$ mg. per cent (6), respectively. Similarly, pretreatment with retrobulbar alcohol to produce denervation did not alter the rise in protein in the aqueous humor. Protein levels 30 minutes after paracentesis were $4,402 \pm 164$ mg. per cent (6) in the alcohol-treated eyes and $4,515 \pm 173$ mg. per cent (6) in control eyes.

In animals pretreated with aspirin, the aqueous humor 30 minutes after paracentesis had a protein level of $1,648 \pm 583$ mg. per cent (5). This observation, that aspirin significantly lowered the protein response to paracentesis, confirmed earlier work. However, the combination of pretreatment with aspirin and denervation with retrobulbar alcohol was not more effective against paracentesis than pretreatment with aspirin alone. The eyes of the former group of animals had a protein rise to $1,279 \pm 217$ mg. per cent (5), not significantly different than eyes of animals of the group treated with aspirin alone.

Discussion
These results demonstrate that the increase in protein in the aqueous humor, after topical nitrogen mustard, is dependent on an intact, sensory innervation. The increase can be partially prevented by retrobulbar anesthesia or trigeminal nerve section, and completely prevented by prior sensory denervation with alcohol. In contrast to the response obtained with exogenous prostaglandins, the nitrogen mustard reaction does not consist of a direct chemical stimulus and response, because it is eliminated by sensory denervation. In all likelihood it is mediated by pain fibers and thus the present findings confirm and extend studies of the irritative ocular response concerning hyperemia, pupillary changes, and changes in intraocular pressure that occur after mechanical stimulation of the iris. Prostaglandins are not media-

![Fig. 3. Effect of retrobulbar lidocaine and alcohol denervation on the breakdown of the blood-aqueous barrier after paracentesis. The two left bars compare the protein levels fifteen minutes after paracentesis in eyes pretreated with saline or retrobulbar lidocaine. Two right bars compare protein levels thirty minutes after paracentesis in control, normally innervated, and alcohol-denervated eyes. No differences are apparent.

Neither is the pathway an adrenergic one, since the response is not influenced by sympathetic denervation.

The current work did not test, and therefore did not specifically exclude, a parasympathetic contribution to the response. Several experiments done with pretreatment of the animals with scopolamine (atropine cannot be used because of atropine-esterase activity in these rabbits), however, did not block either the miosis or the protein increase.

In contrast, the protein response to paracentesis of the anterior chamber is unaffected by sensory denervation but can be prevented by inhibition of the synthesis of prostaglandin. Confirming these results, Miller, Eakins, and Atwal showed PGEl-like activity in the aqueous humor of the rabbit eye after paracentesis and observed that pretreatment with aspirin prevented the appearance of prostaglandins in the aqueous humor. However, prostaglandins do not account for all of the protein re-
response to paracentesis because paracentesis of the anterior chamber of the rabbit still elicits some increase in protein (1,648 mg. per cent, 30 minutes after paracentesis), in spite of inhibition of prostaglandin synthesis. This increased protein in the aqueous humor is apparently not mediated through an extrabulbar neural pathway because sensory denervation induced with alcohol does not prevent the increase. The mechanism of this "basal" protein response to paracentesis is uncertain but may be the result of reflux of blood from the sclera through the trabeculum into the anterior chamber, or vascular leakage from reduction in transmural vascular pressures as the intraocular pressure falls to zero. Other local humoral or reflex factors have not been entirely excluded.

Recently, prostaglandins have been implicated as mediators of irritative responses, particularly paracentesis and iris trauma. The current work shows that although prostaglandins are important, they are not the exclusive mediators that disrupt the blood-aqueous barrier. This observation is consistent with the findings of Cole and Unger that almost no prostaglandin is detectable in the aqueous humor following antidromic trigeminal stimulation or intracameral formaldehyde. Because polyphloretin phosphate (PPP), an antagonist of prostaglandins in other systems, inhibits the hypertensive response to nitrogen mustard or formaldehyde, it has been suggested that this ocular hypertension is prostaglandin-mediated. However, since the response to topical nitrogen mustard requires innervation but is not sensitive to inhibitors of prostaglandin synthesis, and since intracameral formaldehyde elicits no rise in aqueous humor prostaglandin, we feel that the efficacy of PPP in preventing an irritative response is not sufficient evidence for the conclusion that a given reaction is mediated by prostaglandins.

In summary, at least two, and possibly more, pathways can mediate the breakdown of the blood-aqueous barrier in response to injury. The response to paracentesis of the anterior chamber seems to be at least partly mediated by prostaglandins and a neural pathway is probably unimportant. On the other hand, the response to a painful stimulus such as nitrogen mustard is apparently not dependent upon the synthesis or release of prostaglandins, but instead is mediated by nerves. This pathway and its humoral mediators are unknown.

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REFERENCES