It appears that α- and β-adrenergic drugs have effects on the ciliary body in its production of aqueous humor. Beta antagonists 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.17–19 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.

From the Department of Ophthalmology, Mayo Clinic, Rochester, Minn. This study was supported by NEI research grant EY-00634 of the Department of Health, Education, and Welfare, Bethesda, Md.; Research to Prevent Blindness, Inc., New York, N.Y.; The Rowland Foundation, Cambridge, Mass.; and The Mayo Foundation, Rochester, Minn. Submitted for publication Sept. 12, 1979. Reprint requests: Dr. R. F. Brubaker, Mayo Clinic, Rochester, Minn. 55901.

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

<table>
<thead>
<tr>
<th>Ka (min⁻¹ × 10⁻³)</th>
<th>Kc (min⁻¹ × 10⁻³)</th>
<th>Flow (μl/min)</th>
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<td>1.47 ± 0.20</td>
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<tr>
<td>3.67 ± 1.90</td>
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<td>4.63 ± 1.43</td>
<td>15.0 ± 3.16</td>
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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, neurogenicocular 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-
Topical application of nitrogen mustard (NM) to the rabbit eye causes two distinct episodes of ocular hypertension: the first phase, occurring within the first hour, is blocked by prior retrobulbar EtOH-induced "denervation" but not by indomethacin; the second phase, which reaches a peak between 3 and 12 hr after NM application, is blocked by systemic indomethacin treatment but not by denervation. The ocular hypertension, or "breakdown" of the blood-aqueous barrier, during the first hour after topical NM application to rabbit eyes was found to be significantly reduced by prior retrobulbar novocaine, lidocaine, or alcohol injection; section of the ophthalmic division of the fifth nerve; or herpes simplex viral infection. This indicates that the initial ocular response to NM is mediated by a neurogenic mechanism, presumably similar to the axon reflex first described by Bruce, and that the second phase is at least partially mediated by prostaglandins (PGs) or related cyclo-oxygenase products.

The actual chemical mediator of the initial, neurogenic phase has eluded identification. Most of the obvious autacoids have been ruled out since this irritative response could not be blocked by atropine, superior cervical ganglionectomy, systemic H1 or H2 antihistamines, topical scopolamine hydrobromide, or topical or systemic corticosteroids.

To determine whether substance P (SP), a highly vasoactive undecapeptide which is released upon antidromic nerve stimulation from its storage in primary sensory neurons, is involved in the initial NM-induced ocular response, we have used capsaicin (8-methyl-N-vanillyl-6-nonenamide) pre-treatment. Capsaicin, the pungent principle in red pepper, was found to block the response of the rat eye normally produced by topical application of "neurogenic" irritants and to inhibit the increased vascular permeability caused by antidromic stimulation of sensory nerves. More recent evidence indicates that the desensitizing effect of capsaicin is due to its ability to reduce the SP content of primary sensory nerves. SP, or similar peptides, was found to be released into the aqueous humor during trigeminal nerve stimulation, whereas intracameral injection of SP produced a typical irritative response.

Our results demonstrate that capsaicin pre-treatment blocks the initial NM-induced hypertensive response of rabbit eyes. The role of SP in this response is also supported by preliminary observations showing the presence of SP-like immunoreactivity (SP-LI) in the anterior uvea of normal eyes and its accumulation in the aqueous humor after topical NM application.

Materials and methods. New Zealand White rabbits (2 to 4 kg) were placed in rabbit boxes until accustomed to handling and restraint. A 1.0% capsaicin solution was prepared by dissolving 100 mg of capsaicin (Sigma Chemical Co., St. Louis, Mo.) in 150 μl of 100% EtOH; then 850 μl of Tween 80 (Fisher Scientific Co., Pittsburgh, Pa.) was added, followed by 9 ml of saline. Ten or 50 μl of this solution was topically applied to one eye of 14 rabbits; the eyes were rinsed after 3 to 5 min with 2 to 4 ml of saline. The contralateral control eyes were treated with an equal volume of the EtOH–Tween 80–saline mixture or saline and similarly rinsed. After 5 min, a second dose of capsaicin was applied to half of the capsaicin-treated eyes, and half of the control eyes received a second application of saline.

A set of 10 rabbits received unilateral retrobulbar injection of 0.5 ml of 1.0% capsaicin by advancing a 26-gauge 1.0-inch needle through the center of the lower eyelid, near the lid margin, and around the inferior aspect of the globe to a depth of 10 to 20 mm. The ten contralateral eyes served as the controls. In another 6 animals, 0.1, 0.5, or 1.0 ml of 1.0% capsaicin solution was injected intracranially, with an approach similar to the retrobulbar injection except that a longer (1.5-inch) needle was advanced its full length through the superior orbital fissure. IOPs of conscious animals were measured with a Pulsair (Fort Dodge Laboratories, Fort Dodge, Iowa) before retrobulbar or intracranial injections. IOPs of conscious animals were measured with a...
Fig. 1. Frequency of effective blockage of NM-induced initial hypertension 6 hr to 4 days after topical, retrobulbar, or intracranial capsaicin administration and its comparison to the efficacy of EtOH denervation. Criteria for effective blockage: maximum IOP rise during first hour after NM application ≤5 mm Hg from either baseline or contralateral control IOP, and ≤3 mm Hg from both. Number of eyes in parentheses.

pneumatic floating-tip tonometer (calibrated on cannulated rabbit eyes) after topical application of 1 drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories, Inc., Fort Worth, Texas). Pupillary diameters were measured with a pupil gauge.

Five hours to 4 days after the last capsaicin administration, pupillary and corneal reflexes were tested, and baseline measurements of IOP and pupillary diameter were obtained on both eyes of each rabbit prior to unilateral or bilateral topical application of 50 μl of 1.0% NM (mechlorethamine hydrochloride, Sigma) in normal saline to the cornea. Fifty microliters of normal saline were similarly applied to control eyes. All eyes were rinsed 3 to 5 min later with 2 to 4 ml of saline. IOP and pupillary diameter measurements were taken at 10 min intervals during the first 40 min after NM application, and eyes were observed for several days for signs of ocular inflammation.

Another group of 10 normal rabbits, eight of which were treated unilaterally with 1.0% NM, were killed with an overdose of sodium pentobarbital. Aqueous was removed and pooled from groups of four identically treated eyes: aqueous from four eyes taken 30 min after unilateral NM application (pooled sample 1) and the aqueous taken from the contralateral eyes of these rabbits (pooled sample 2); aqueous taken from four eyes 60 min after unilateral NM application (pooled sample 3) and aqueous taken from the contralateral eyes of these rabbits (pooled sample 4); and aqueous taken from four eyes of two normal, untreated rabbits (pooled sample 5). One milliliter of 4N acetic acid was added to 1 ml of each of the five pooled samples. Pooled samples 2, 4, and 5 represented controls; 5 μl of a 1.0% NM solution was added to pooled sample 4 to determine whether the presence of NM affects the immunoassay. The iris-ciliary body was also removed from these eyes and similarly pooled into five groups, each containing four tissues. The SP-LI in these pooled aqueous and anterior uvea samples was determined by radioimmunoassay, through the courtesy of Dr. S. E. Leeman of Harvard Medical School.

Results and discussion. Topical, retrobulbar, or intracranial capsaicin administration produced immediate pain, as evidenced by lid-squeezing and vocalization. Vocalization was not effectively inhibited by general anesthesia. Conjunctival swelling and hyperemia occurred within 10 min after capsaicin administration. The chemosis and conjunctival hyperemia resolved within 6 hr after topical and within 1 day after retrobulbar or intracranial administration.

The IOP was measured, and the eyes were examined 5 to 6 hr after capsaicin administration and/or 0.5 to 1 hr before topical NM application. At these times none of the typical signs of uveitis...
was apparent regardless of the route of capsaicin administration. Eyes that received topical capsaicin had an IOP of 21.2 ± 0.6 mm Hg as compared to the contralateral IOP of 22.2 ± 0.5, and both eyes had normal and identical pupillary diameters (6.2 ± 0.4 mm). Following retrobulbar capsaicin injection the mean IOPs were 19.0 ± 0.3 mm Hg vs. 20.0 ± 0.6 for treated and untreated eyes, respectively; the pupils were also normal (6.7 ± 0.3 mm). Animals that received intracranial capsaicin injection had their eyes first examined 1 to 3 days after treatment (within 1 hr before NM application); again no signs of uveitis were apparent. The mean IOPs were 19.3 ± 0.7 mm Hg and 19.0 ± 1.2 for OD and OS, respectively, and the mean pupillary diameters of all these eyes were 6.3 ± 0.3 mm. All these values were within the range obtained routinely on untreated rabbits in our laboratory under identical conditions. These observations clearly show that the capsaicin-treated eyes did not have an ongoing intraocular inflammation at the time of the NM application.

Topical capsaicin pretreatment produced an effective blockage of the NM-induced initial hypertensive response in only about half of the rabbit eyes (Fig. 1). In contrast, either retrobulbar or intracranial capsaicin pretreatment proved effective in blocking the initial hypertensive response of the rabbit eye to topically applied NM (Fig. 1). In all eight eyes of four rabbits which received intracranial capsaicin and in three out of four eyes that received retrobulbar capsaicin pretreatment 1 to 3 days before NM application, the initial hypertensive phase of the NM response was effectively blocked. These findings compare favorably to the percentage of eyes effectively blocked 2 to 3 weeks after retrobulbar EtOH injection. The fact that a single or two consecutive topical capsaicin applications did not cause a complete desensitization to the initial ocular irritative effects of NM is not surprising, since Szolcsányi and Jancsó-Gábor13 found that as many as 10 consecutive topical capsaicin applications were required to obtain complete desensitization of the rat eye. Rabbits are reputed to be less sensitive to the desensitizing effects of capsaicin (A. Jancsó-Gábor, personal communication). For this reason and because of the much greater body weight of rabbits, systemic capsaicin application, which is very effective in rats, was judged to be costly and impractical.

It should be noted that although topical or retrobulbar capsaicin administration had a unilateral effect, intracranial capsaicin administration blocked the NM effect on both eyes. Retrobulbar or intracranial capsaicin injection 1 to 3 days before NM application was also found to be at least as effective as retrobulbar EtOH denervation when the data were analyzed in terms of the mean extent of the peak IOP change during the first hour after NM application (Fig. 2). Capsaicin pretreatment also proved equally effective as retrobulbar EtOH denervation1 in inhibiting the initial miotic response following NM application. Capsaicin pretreatment, like EtOH denervation, did not block the long-term cytotoxic effects observed several days after NM application.

Capsaicin pretreatment has many advantages over retrobulbar EtOH administration. The capsaicin-induced ocular irritative response was less severe and much shorter in duration than the retrobulbar EtOH-induced ocular inflammation, which lasts for 1 to 2 weeks. Most importantly, unlike EtOH-denervated eyes, all capsaicin-treated eyes had normal pupillary and corneal reflexes. This is consistent with the observation that capsaicin does not cause extensive degeneration of primary afferent terminals in adult animals.

Clearly, capsaicin is a much more selective agent.
than EtOH because it apparently eliminates the neurogenic mediator of this response without eliminating functional innervation.

All three pooled samples of normal rabbit anterior uvea, each consisting of the iris-ciliary body taken from four untreated eyes, showed substantial SP-LI (7.9 ± 0.3 x 10⁻¹⁵ mol/mg wet weight), comparable to levels found in the rat cerebral cortex.¹⁵ The single pooled sample of four anterior uvea taken 1.0 hr after NM application showed an approximately 50% reduction in SP-LI. Thirty or 60 min following unilateral topical application of 1.0% NM, SP-LI in the aqueous humor was increased approximately threefold over that in contralateral control aqueous samples, which itself contained high (8.0 to 9.0 x 10⁻¹⁵ mol/ml of aqueous) than normal levels (nondetectable). NM added to aqueous samples did not affect the radioimmunoassay. These preliminary observations, together with the finding that intracameral injection of SP reproduces some of the signs of ocular irritation, are consistent with the hypothesis that the initial ocular hypertensive and the miotic response to chemical irritants, play an important role and may, in fact, be the primary mediators of the initial ocular response to chemical irritants. Capsaicin provides a more specific experimental tool than denervation for separating and identifying the neurogenic component of inflammatory responses. Furthermore, pretreatment with capsaicin congeners, which are more potent desensitizers but less dolorific than capsaicin,¹³ may prove beneficial in blocking non-PG-mediated irritative responses. Combined pretreatment with such neurogenic desensitizers and PG synthesis inhibitors can therefore be expected to block both the initial and the second phase¹⁶ of the pathophysiological response of the eye to chemical irritants and/or trauma.

From the Department of Ophthalmology, Research Division, Columbia University College of Physicians & Surgeons, New York, N.Y. This investigation was supported by USPHS Research Grant EY 00333 from the National Eye Institute. Presented at the Annual National Meeting of ARVO, April 1979. Submitted for publication May 30, 1979. Reprint requests: Dr. L. Z. Bito, Department of Ophthalmology, Research Division, Columbia University, 630 West 168th St., New York, N.Y. 10032.

Key words: substance P, capsaicin, nitrogen mustard, uveitis, ocular inflammation, irritation, denervation, eye, intraocular pressure, miosis

REFERENCES


An implantable system is described which continuously delivered an aqueous solution to the external surface of six rabbit eyes for 6 weeks. A polytetrafluoroethylene tube was implanted in the superior conjunctival fornix 4 weeks prior to the implantation of the Infusaid pump. The pump provides a fluid source which is easily refilled and requires no batteries or external power source.

The continuous delivery of drugs to the ocular and periorcular tissues is useful in pharmacological studies in rabbits. The recently developed Alzet osmotic minipumps are self-powered implantable pumps which deliver solutions of experimental agents to the rabbit eye at controlled rates for periods of up to 1 week. Experiments of longer duration require replacement of the minipumps, which entails a risk of inflammation and infection at the pump sites. We describe herein an implantable closed pump and tube system that has been devised for the continuous delivery of aqueous solutions to the external rabbit eye for at least 6 weeks.

Materials and methods. The pump used in this system is the Infusaid manufactured by Metal Bellows Corp. (Fig. 1). This pump was developed and is being used for long-term anticoagulation therapy.2–4 The pump is a two-chambered device, with one chamber containing the drug in aqueous solution and the other containing the charging fluid. The charging fluid is a fluorocarbon in liquid-gas equilibrium which is altered by body temperature. The driving force of the expanding charging fluid at body temperature and the fixed outflow resistance combine to provide a constant flow rate for an aqueous solution.

The pump currently available is the Model 100, which has a drug volume of 47 ml, a diameter of 86 mm, a thickness of 27 mm, an empty weight of 189 gm, and a rate of delivery of 1 ml/day. Small pumps, with a volume of 5 ml, are being developed. The smaller pumps should be better suited to animal experimentation than the larger models described in this paper.

An expanded polytetrafluoroethylene (PTFE) tube was designed in cooperation with Gore Associates for externalization of the system in the superior conjunctival fornix. The PTFE tube consists of an outer surface containing interspaces, which is compact enough to prevent further tissue ingrowth and occlusion of the lumen. Larger-diameter tubes of expanded PTFE have been used in vascular surgery for venous and arterial grafts.5–10

The PTFE tube was implanted in the superior conjunctival fornix of six rabbits 4 weeks prior to the implantation of the pump. A location in the superior conjunctival fornix was chosen for insertion of a metal cannula. The cannula was directed subcutaneously from the superior fornix to an exit site over the occipital region. The PTFE tube was drawn into the metal cannula, the cannula was then withdrawn through the exit site, leaving the PTFE tube in place. The PTFE tube was sutured to the conjunctiva with 7.0 Prolene suture so that the external orifice was 1 to 2 mm beyond the plane of the conjunctiva. Previous experiments in which the external orifice of the PTFE tube was positioned more than 2 mm beyond the plane of the conjunctiva resulted in extrusions, whereas placement of the orifice in the plane of the conjunctiva resulted in retractions. The PTFE tube was then anchored posteriorly to the periosteum over the occipital region with 4.0 prolene. The exit site in the occipital region was also closed with 4.0 prolene. The tube was allowed to remain undisturbed for a period of 4 weeks prior to the implantation of the pump so that fibrous tissue ingrowth would secure the tube firmly to the surrounding tissues. Previous experiments in which the PTFE tube and pump were implanted simultaneously resulted in retraction or extrusion of the PTFE tubes in the superior conjunctival fornix.

The Infusaid pump was implanted in a subcutaneous pocket in the lumbar region of the six rabbits 4 weeks after placement of the PTFE tube. The silicone catheter attached to the outlet of the pump was passed subcutaneously and connected to the PTFE tube in the occipital region. The incision sites in both the occipital and lumbar regions were closed with 4.0 prolene suture.

It was necessary to refill the pump every 4 weeks. This was accomplished by passing a 22-gauge Huber point needle transcutaneously and piercing the inlet septum of the pump. Solution of filtered 0.9% sodium chloride and 0.5% fluores-