example, these ionophores induce secretion or release of histamine from mast cells and enzymes such as amylase from exocrine pancreas, modulate sensitivity of neuromuscular junctions, and mimic the effect of phytohemagglutinin on lymphocyte transformation.1

X537A together with calcium increases acetylcholine release elicited by nerve stimulation4 and A23187 causes a calcium-dependent release of catecholamines from perfused cat adrenal glands.2 X537A complexes and can transport epinephrine,3 and A23187 causes potassium release from rat parotid slices, simulating the action of epinephrine on the &alpha;-adrenergic receptor.7 Thus release, transport, and action of neurotransmitters can be induced by ionophores.

Another relevant model is the fly salivary gland in which ionophore A23187 stimulates fluid secretion and increases calcium efflux and influx.2 This effect requires external calcium and does not increase cyclic AMP. In this gland, 5-hydroxytryptamine increases cyclic AMP, fluid secretion, involving a potassium pump, and passive chloride movement, also requiring calcium as a messenger.2 Thus, there is precedent for a role of calcium and an effect of calcium ionophores in the secretion of fluid and ions. Of note, acetazolamide reduces the flux of chloride across isolated gastrointestinal epithelium.6 The mechanism by which this drug lowers intraocular pressure is not completely clear. One may question a possible effect of acetazolamide on calcium movement.

Little is known about the effect of these ionophores and calcium on ocular function. X537A induces the release of neurotransmitters, taurine, glycine, and &gamma;-aminobutyrate, accompanied by increased calcium ion uptake, in chick retina.9 In the retina, inhibition by light of guanylate cyclase may relate to light-induced release of calcium from disk membranes. In the anterior segment, the role of calcium is unclear. It may be involved in the actions of certain agents on the eye. For instance, prostaglandin E2, which elevates intraocular pressure, may act as a calcium ionophore.10 Obviously, the interaction of the cyclic nucleotides, prostaglandins, and calcium, and other ions in aqueous humor secretion requires much further elucidation and the action of the ionophores may be a valuable probe in this regard.

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Key words: intraocular pressure, calcium, cation ionophores A23187 and X537A.

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Potentiation of the effects of topical epinephrine on the pupil and intraocular pressure in the sympathetically denervated rabbit eye by a catechol-O-methyl transferase inhibitor. LARRY P. BAUSHER AND MARVIN L. SEARS.

Dose-response curves of increase in pupil size and decrease in intraocular pressure with topical epinephrine have been determined in the sympathetically denervated rabbit eye. Topical pretreat-
ment with the catechol-O-methyl transferase inhibitor U-0521 potentiated the effects of epinephrine on both the pupil and pressure. These observations suggest a possible role for catechol-O-methyl transferase in the aqueous humor dynamics of the supersensitive eye. The possible use of the denervated rabbit eye as an experimental model for the glaucomatous eye in evaluating the ocular effects of adrenergic agents is discussed.

Although the influence of adrenergic drugs and the sympathetic nervous system upon aqueous humor dynamics is well established, many details concerning the functions of the adrenergic system of the eye and mechanisms controlling it are not understood. Recent studies have suggested a possible role for monoamine oxidase in aqueous humor dynamics, but the role of catecholamine-metabolizing enzymes in the regulation of intraocular pressure (IOP) or the termination of action of catecholamines in the eye has not been systematically studied. In this communication we report the potentiation by the catechol-O-methyl transferase (COMT) inhibitor U-0521 (3',4'-dihydroxy-2-methyl propiophenone) of the increase in pupil size and IOP lowering induced by epinephrine (EPI) in the denervated rabbit eye.

Methods. Six albino rabbits received right superior cervical ganglionectomy while under sodium pentobarbital anesthesia. After 2 to 7 weeks they were used for studies of pupil diameter. Experiments were conducted in an ordinary laboratory under constant fluorescent illumination. Three animals at a time were wrapped gently to restrain movement and pupil diameters were estimated to the nearest 0.5 mm. with a 6 inch rule. Each measurement consisted of an average of three readings taken at approximately 3 to 5 min. intervals. Two drops of 1-epinephrine-dibitartrate (Schwartz/Mann Division, Becton, Dickinson & Co., Orangeburg, N. Y.) dissolved in 0.9 per cent NaCl were layered on the cornea of both eyes and pupil measurements were begun 15 min. later. In potentiation experiments, 2 drops of a solution of U-0521 in NaCl were layered onto both eyes and pupil diameters were similarly recorded 15 min. later. At 45 min. after inhibitor applications, EPI was applied and the increase in pupil size determined 15 min. later.

Effects of EPI on pressure in the denervated eye were studied 10 to 27 weeks after ganglionectomy. Pressures were measured hourly from 10:00 A.M. to 4:00 P.M. EPI was applied as above at 11:00 A.M. in potentiation experiments, a total of 1.38 mg. of U-0521 was applied as above in three equal 0.46 mg. portions at 10:00, 11:00, and 12:00. Rabbits were wrapped one at a time for each measurement and between readings were unwrapped and kept in individual cages to avoid undue excitement. Mean pressures, recorded to the nearest whole millimeter of mercury, were estimated after propanocaine anesthesia by observing the visual display of an Alcon Pneumotonomograph (PTG) and averaging the readings of three or four successive applanations. For graphical presentation of the dose-response curves, PTG readings were converted to true IOP's with a linear regression equation calculated for a calibration curve, determined by Dr. Eugenio Maul (personal communication). Outflow pressures were then calculated by subtracting an assumed value of 10 mm. Hg for the episcleral venous pressure.

Results.

Pupil response. The log dose-response curve for the increase in pupil size in the denervated eye 15 to 30 min. after topical epinephrine is shown in Fig. 1. The threshold response occurred at about 12 µg of EPI. By qualitative observation the responses appeared to be maximal 15 min. after

<table>
<thead>
<tr>
<th>EPI*</th>
<th>U-0521+Saline</th>
<th>U-0521+EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denervated eye</td>
<td>0.8 ± 0.2 (6)</td>
<td>0.3 ± 0.1 (5)</td>
</tr>
<tr>
<td>Normal eye</td>
<td>0.2 ± 0.1 (6)</td>
<td>0.02 ± 0.08 (5)</td>
</tr>
</tbody>
</table>

*No pretreatment with U-0521.
†Mean ± S.E.M.; number of observations are in parentheses.

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Fig. 2. Log dose-response curves for decrease in outflow pressure in the denervated eye 3 hr. after topical EPI, with and without pretreatment with U-0521. Values are expressed as mean ± S.E.M. (n is given in parentheses).

after application of EPI. No systematic changes in pupil size were observed during the 10 to 15 min. period required for the measurement of both pupils of three animals. Significant responses were not observed in the contralateral normal eye with either saline or as much as 94 μg of EPI.

Potentiation of the pupil response was investigated by applying 570 μg of U-0521, followed by 12 μg of EPI 45 min. later, and measuring pupil size 15 to 30 min. after both inhibitor and EPI. Significant potentiation with U-0521 pretreatment was observed in the denervated eye but not in the normal eye (Table I). Controls in which saline was substituted for EPI showed insignificant increases in the denervated eye.

Pressure response. A typical time course of the mean pressure response in the denervated eyes of three of the four rabbits showed a dose-dependent rise in IOP 1 hr. after EPI, followed by a decrease to about baseline pressures after 2 hr. A maximal decrease in pressure occurred at 3 hr., followed by gradual recovery, which was still incomplete at 5 hr. The fourth animal consistently failed to show the increase in pressure after 1 hr. and also showed a greater decrease after 3 hr. Norton and Vierstein's have reported similar observations of a transitory pressure rise after topical EPI in 75 per cent of normal rabbit eyes. The normal eyes, which received saline instead of EPI, consistently showed a decrease in pressure 1 hr. after the first pressure measurement, and remained hypotensive throughout the experimental session.

The hypotensive response in normal eyes was also observed in control experiments, in which both eyes received saline, whereas no significant pressure variations were observed in the denervated eye throughout the 6 hr. experiment period. At 10:00 A.M., pressures in both eyes were not significantly different. After 10:00 A.M., pressures in the normal eye varied little but were significantly lower than those in the denervated eye. The general appearance of the control curves was unaffected by pretreatment with U-0521.

Log dose-response curves for the decrease in outflow pressure in the denervated eye 3 hr. after EPI, with and without pretreatment with U-0521, are shown in Fig. 2. Without inhibitor, 50 per cent of maximal decrease in pressure occurred with about 4.3 μg of EPI, and the maximal response was 5.2 mm. Hg. In the presence of U-0521, 50 per cent of maximal decrease occurred at about 0.9 μg of EPI, and the maximal response was 3.2 mm. Hg. Dose-response curves of the pressure decrease 4 and 5 hr. after EPI and the transitory pressure increase after 1 hr. showed patterns similar to that in Fig. 2.

Discussion. Dose-response curves of the changes in pupil size and pressure of the surgically denervated rabbit eye after topical EPI have not been reported previously. Lamble's has reported a dose-response curve of topical EPI on maximal IOP decrease in the normal rabbit eye, which occurred 4 to 5 hr. after EPI application, and a value of about 170 μg of EPI can be calculated as giving the 50 per cent of maximal response from his data. The pressure decrease of the denervated eye therefore is about 40 times more sensitive to topical EPI than that of the normal eye. The shift to the left of the pressure dose-response in the denervated eye in the presence of U-0521 suggests that intraocular COMT might
be involved in the inactivation of EPI. This hypothesis is supported by enzyme data in vitro, which showed that the inhibitor acts as a competitive substrate with EPI for a partially purified COMT preparation from the rabbit iridal-ciliary body (L. P. Bausher, unpublished observations). Irid-ciliary body COMT activity is unaffected by sympathetic denervation, and in most peripheral tissues COMT appears to have a predominantly extra-neuronal location.

It is widely accepted that the principal mechanism of inactivation of catecholamines at the neuroeffector junction in most varieties of normal smooth muscle involves reuptake into the pre-junctional neuron and binding in intraxonal storage granules. Enzymatic inactivation processes are generally found to be unimportant. However, some studies in vitro using tissues which are highly sensitive to catecholamines as a result of prior denervation or treatment with cocaine, suggest a possible role for COMT in the termination of action. Wylie and co-workers have demonstrated potentiation in vivo of the effects of O-methyl transferase inhibitors on blood pressure. Knowledge of the mechanism of the termination of action of EPI in lowering IOP in glaucomatous eyes could be helpful in elucidating the role of the adrenergic system in aqueous humor dynamics. Nerve degeneration in the trabecular region has been reported in open-angle glaucoma and in the human aging process. In the absence of uptake into nerve endings, both denervated and glaucomatous eyes might respond similarly to EPI and drugs which potentiate the effects of EPI. Further clinical and experimental study in this area may be useful.

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Key words: catechol-O-methyl transferase inhibition, aqueous humor dynamics, intraocular pressure, sympathetically denervated eye, epinephrine, in vivo potentiation, dose-response curves, U-0521.

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6-Aminohexanoic acid is a potent antifibrinolytic agent with the ability to retard dissolution of fibrin clots. The corneal penetration of 6-aminohexanoic acid was measured to determine if therapeutic levels of the drug could be maintained in the anterior chamber by topical administration. The conjunctival sac to anterior chamber transfer coefficient was found to be 1.51 x 10^-4 ± 0.20 x 10^-4 per minute and the corneal permeability coefficient found to be 3.46 x 10^-5 ± 0.46 x 10^-5 cm. per second in the rhesus monkey eye. The results indicate that, to establish a therapeutic level in the aqueous humor, a high concentration of drug would have to be used with topical drop administration or a zero order delivery system must be used.

In a prospective, randomized clinical trial, 6-aminohexanoic acid (episolon-aminocaproic acid)