copithecus ethiops under pentobarbitone anesthesia, whereas a value of 20 mm. Hg was found in the same species under light ether anesthesia. Kitazawa and Langham have reported values of 18-20 mm. Hg in Macaca mulatta sedated with phencyclidine. Thus, in view of the finding of Cevario and Macri that pentobartibarbite apparently reduces aqueous inflow, it is not considered surprising that the intraocular pressure has been found to be higher in conscious owl monkeys than in those anesthetized with pentobarbitone.

This study has demonstrated that the intraocular pressure of owl monkeys is reduced in a dose-related manner by several parasympathomimetic agents. Oxotremorine was the most potent compound tested and its effects were prolonged compared with the other drugs. It has been demonstrated to have a higher heptane:water partition coefficient than either pilocarpine (71 times) or R.S. 86 (12 times), and this suggests that it would penetrate membranes more easily, which might contribute to its greater potency.

Pilocarpine gives maximal reductions in intraocular pressure in many glaucoma patients at a concentration of 2.0 per cent, and it has been shown to have similar potency in the owl monkey. However, the duration of its effects in human beings has been reported to be greater than that which was observed in the monkeys. Carbachol has been shown to have slightly greater potency and duration of effects than pilocarpine in man, provided that a surfactant is incorporated in the solution. In the owl monkey slightly lower potency was found compared to pilocarpine, although there was no surfactant in the solution. Aceclidine has been reported to have similar potency to pilocarpine in man, but it was markedly less potent in the owl monkey.

In conclusion, it may be said that the fully conscious owl monkey responds to locally applied parasympathomimetic drugs with a pattern of changes in intraocular pressure which is not dissimilar to that seen in human beings. The species would, therefore, possibly represent a valuable predictive model for the investigation of new parasympathomimetic glaucoma treatments.

We thank Mr. E. J. McQuade of Digilab, Inc., for the gift of the floating probe and Dr. M. Taeschler of Sandoz for supplying the R.S. 86. We are also grateful to Mrs. J. Barrett for technical assistance.


Key Words: owl monkey, Aotus tricoloratus, tonometry, intraocular pressure, parasympathomimetics, pilocarpine.

REFERENCES


The effect of cation ionophores on intraocular pressure. STEVEN M. PODOS.

The ionophores A23187 and X537A, which increase the permeability of cell membranes to calcium and other divalent cations, produced significant elevation of intraocular pressure in rabbits. Topical instillation of these ionophores in concentrations of 1.0 and 0.1 per cent were effective. Aqueous humor protein and facility of outflow were similar in ionophore-treated and control eyes. Pretreatment with indomethacin did not block the intraocular pressure rise induced by A23187. Alterations of intracellular calcium might control cellular processes within the eye as in many other biological systems.

The secondary messenger system of cyclic nucleotides is involved in the control of aqueous humor dynamics. Changes in the cyclic adenosine 3',5'-monophosphate (cyclic AMP) level in aque-
baseline intraocular pressure was measured with

0.5 per cent proparacaine.

This 1.0 per cent milky suspension was diluted
adding 10 mg. to a diluent of 0.1 ml. of di-
methylsulfoxide (DMSO) and 0.9 ml. of water.

centrations of ionophore.

The ionophores are lipophilic antibiotics which com-
plex various cations and facilitate their transport
across a variety of membranes. The

Table I. Effect of ionophore A23187 on intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No.</th>
<th>0 min.</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>240 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0%</td>
<td>8</td>
<td>23.4 ± 1.4</td>
<td>31.4 ± 1.3*</td>
<td>32.1 ± 1.4*</td>
<td>30.6 ± 1.1</td>
<td>21.5 ± 1.0</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>23.8 ± 1.3</td>
<td>27.1 ± 1.4</td>
<td>23.3 ± 1.7</td>
<td>26.3 ± 1.4</td>
<td>24.1 ± 1.4</td>
</tr>
<tr>
<td>0.1%</td>
<td>8</td>
<td>23.9 ± 0.6</td>
<td>30.3 ± 1.3</td>
<td>32.1 ± 1.2*</td>
<td>28.5 ± 1.5</td>
<td>21.9 ± 1.1</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>24.1 ± 0.8</td>
<td>25.9 ± 1.0</td>
<td>26.8 ± 1.0</td>
<td>26.7 ± 1.6</td>
<td>23.6 ± 1.1</td>
</tr>
<tr>
<td>0.01%</td>
<td>8</td>
<td>21.4 ± 0.6</td>
<td>21.8 ± 0.8</td>
<td>24.5 ± 0.4</td>
<td>22.5 ± 0.6</td>
<td>20.5 ± 1.2</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>21.6 ± 0.7</td>
<td>21.6 ± 0.7</td>
<td>23.5 ± 0.5</td>
<td>22.5 ± 0.5</td>
<td>21.3 ± 1.3</td>
</tr>
</tbody>
</table>

*Significant difference between eye treated with ionophore and fellow eye, paired-t test, p < 0.02.
†Significant difference between eye treated with ionophore and fellow eye, paired-t test, p < 0.01.

Table II. Effect of ionophore X537A on intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No.</th>
<th>0 min.</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>240 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0%</td>
<td>8</td>
<td>20.3 ± 0.9</td>
<td>28.6 ± 1.8*</td>
<td>27.1 ± 1.9*</td>
<td>24.5 ± 2.1</td>
<td>20.5 ± 1.8</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>20.0 ± 1.0</td>
<td>18.3 ± 1.1</td>
<td>20.8 ± 1.1</td>
<td>19.4 ± 1.5</td>
<td>18.1 ± 1.2</td>
</tr>
<tr>
<td>0.1%</td>
<td>8</td>
<td>21.2 ± 0.8</td>
<td>26.1 ± 0.8*</td>
<td>24.9 ± 1.3</td>
<td>28.4 ± 1.6</td>
<td>21.0 ± 1.1</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>21.0 ± 1.2</td>
<td>22.4 ± 1.2</td>
<td>23.5 ± 1.4</td>
<td>25.1 ± 1.3</td>
<td>21.5 ± 1.2</td>
</tr>
<tr>
<td>0.01%</td>
<td>8</td>
<td>20.6 ± 1.4</td>
<td>21.5 ± 1.4</td>
<td>23.4 ± 1.1</td>
<td>22.8 ± 0.9</td>
<td>19.9 ± 0.7</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>20.8 ± 1.4</td>
<td>20.4 ± 1.3</td>
<td>21.1 ± 1.4</td>
<td>22.3 ± 1.1</td>
<td>19.8 ± 1.0</td>
</tr>
</tbody>
</table>

*Significant difference between eye treated with ionophore and fellow eye, paired-t test, p < 0.005.

ous humor are associated with alterations of intra-
ocular pressure induced by catecholamines and
prostaglandins.

Calcium also is an important intracellular mes-
senger, often interacting with the cyclic nucleo-
tides to control physiological processes. The
ionophores are lipophilic antibiotics which com-
plex various cations and facilitate their transport
across a variety of membranes. X537A and
A23187 are diverant cation ionophores which in-
crease the permeability of cell membranes to cal-
cium. X537A also complexes monovalent cations
and forms complexes with organic amines such as
epinephrine.

Many cell processes are stimulated by these
ionophores, including some types of fluid and
enzyme secretion, release of hormones and neural
transmitters, and contraction of muscle. The
following study describes the effect of topical ap-
lication of the ionophores A23187 and X537A on
the intraocular pressure of rabbit eyes.

Methods. Ionophores A23187 (mol. wt. 523) and
X537A (mol. wt. 561) were prepared by adding
10 mg. to a diluent of 0.1 ml. of di-

methylsulfoxide (DMSO) and 0.9 ml. of water.
This 1.0 per cent milky suspension was diluted
with water to produce 0.1 and 0.01 per cent con-
centrations of ionophore.

Awake albino rabbits (weighing 2 to 3 kilo-
grams) were restrained in canvas wraps. After
topical anesthesia with 0.5 per cent proparacaine,
baseline intraocular pressure was measured with
an Alcon applanation pneumotonomograph that had
been manometrically calibrated. In each experi-
ment 0.05 ml. of each ionophore in one of the
above concentrations was applied to one cornea
and 0.05 ml. of its appropriate diluent to the
fellow eye. Equal numbers of right and left eyes
were treated with drug. Intraocular pressure
measurements were repeated at 15, 30, 60, 120,
and 240 minutes and 6 hours after application of
the drug.

In other animals, baseline tonography was
performed on day 1 with an Alcon EDT-103
tonomography unit. One eye was treated with 0.05
ml. of 0.1 per cent A23187 and the fellow eye
with 0.05 ml. of diluent in each rabbit on day 2.
Tonography was carried out 1 hour later.

In similar experiments baseline intraocular pres-
sure was measured and 0.05 ml. of 0.1 per cent
A23187, 0.1 per cent X537A, or 1.0 per cent
X537A was instilled in one eye and diluent in the
other. Thirty minutes later, pressure was
measured and anterior chamber paracentesis
done to remove aqueous humor for measurement of
protein.

Equal numbers of rabbits were pretreated with
indomethacin, 10 mg. per kilogram intraperitoneal-
ly, or an equal volume of diluent. One hour later,
intraocular pressure was measured and 0.05 ml. of
each ionophore in one of the
fellow eye. Equal numbers of rabbits were pretreated with
indomethacin, 10 mg. per kilogram intraperitoneal-
ly, or an equal volume of diluent. One hour later,
intraocular pressure was measured and 0.05 ml. of
each ionophore in one of the
bits, one eye was pretreated with 1 drop of prednisolone acetate 1.0 per cent and the fellow eye with an equal volume of water. Thirty minutes later both eyes were treated with 0.05 ml. of 0.1 per cent A23187 and intraocular pressure measured.

Statistical analyses employed the paired-t test. Differences of p < 0.02 were considered significant.

Results. Ionophore A23187 produced an elevation of intraocular pressure in rabbits (Table I). At 60 minutes after application of the 1.0 per cent solution, the mean intraocular pressure was significantly (p < 0.01) higher in the eyes treated with ionophore than in the diluent-treated fellow eyes. The 0.1 per cent A23187 also elevated intraocular pressure with a peak at 60 minutes (p < 0.01). There was no significant difference between the outflow facilities of seven ionophore-treated eyes, 0.28 ± 0.04 µl per minute per millimeter of mercury, and the diluent-treated eyes, 0.31 ± 0.05 µl per minute per millimeter of mercury, at this time. Intraocular pressure returned to normal by 240 minutes. Ionophore X537A induced an elevation of intraocular pressure in concentrations of 1.0 and 0.1 per cent (Table II) which appeared to peak at about 30 minutes (p < 0.005) after instillation. Mild conjunctival redness was seen in some eyes treated with either compound at the 1.0 and 0.1 per cent concentrations and with DMSO alone. Slit-lamp examination revealed no cells or flare. X537A, 0.1 and 1.0 per cent, occasionally produced some cloudiness of the corneas from 60 to 240 minutes, whereas the 0.01 per cent solution did not.

In seven rabbits at 30 minutes after the administration of 0.1 per cent A23187 the intraocular pressure was significantly (p < 0.01) elevated whereas mean aqueous humor protein was not significantly different (p > 0.1), in a comparison of the eyes treated with ionophore and fellow eyes treated with diluent (Table III). X537A, 0.1 and 1.0 per cent, also produced intraocular pressure elevation in the absence of a significant aqueous humor protein rise (Table III).

Pretreatment with indomethacin, 10 mg./kg., did not block the effect of A23187, 0.1 per cent, on intraocular pressure (Table IV). The pressure response of eyes pretreated prior to ionophore with topically administered 1.0 per cent prednisolone acetate was not significantly different from that of fellow eyes pretreated with diluent. In some animals in these experiments no rise of intraocular pressure was noted in either eye. Both anti-inflammatory agents appeared to reduce the conjunctival redness.

Comment. The ionophores A23187 and X537A elevate intraocular pressure when applied topically to rabbit eyes. This effect appears to involve increased production of aqueous humor since outflow facility is not altered by A23187. Since variable conjunctival irritation and corneal clouding occur after instillation of effective concentrations of these ionophores, an inflammatory response may account for the pressure rise. Although there is a trend in one direction, aqueous humor protein levels are not elevated significantly by these ionophores given in a concentration that produces an inflammatory response may account for the pressure rise. Moreover, anti-inflammatory agents such as indomethacin, an inhibitor of prostaglandin synthesis, and prednisolone do not block the rise of intraocular pressure. A neural pathway for disruption of the blood-aqueous barrier, not amenable to therapy with nonsteroidal anti-inflammatory compounds, also seems unlikely, since this would be expected to alter aqueous humor protein.

Although A23187 and X537A complex a variety of ions, their effect on calcium transport is of greatest interest because of the evidence that calcium acts as another secondary messenger and interacts with the cyclic nucleotides. Calcium is involved in many cellular processes that may affect aqueous humor dynamics. The ionophores enhance these physiological processes by increasing calcium permeability and redistributing calcium from intracellular storage compartments. For

Table III. Effect of ionophores on aqueous humor protein in rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intraocular pressure mean (mm. Hg) ± S.E.</th>
<th>Aqueous protein mean (mg./ml.) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
<td>30 min.</td>
</tr>
<tr>
<td>A23187, 0.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluent</td>
<td>15</td>
<td>21.3 ± 0.8</td>
</tr>
<tr>
<td>X537A, 0.1%</td>
<td>7</td>
<td>22.3 ± 1.3</td>
</tr>
<tr>
<td>Diluent</td>
<td>7</td>
<td>22.9 ± 1.2</td>
</tr>
<tr>
<td>X537A, 1.0%</td>
<td>8</td>
<td>22.1 ± 1.0</td>
</tr>
<tr>
<td>Diluent</td>
<td>8</td>
<td>21.8 ± 1.0</td>
</tr>
</tbody>
</table>

*Significant difference between eye treated with ionophore and fellow eye, paired-t test, p < 0.01.

Table IV. Effect of indomethacin on ionophore A23187–induced elevation of intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean intraocular pressure (mm. Hg) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>Pretreated with indomethacin, 10 mg./kg.:</td>
<td></td>
</tr>
<tr>
<td>Ionophore, 0.1%</td>
<td>7</td>
</tr>
<tr>
<td>Diluent</td>
<td>7</td>
</tr>
<tr>
<td>No indomethacin:</td>
<td></td>
</tr>
<tr>
<td>Ionophore, 0.1%</td>
<td>6</td>
</tr>
<tr>
<td>Diluent</td>
<td>6</td>
</tr>
</tbody>
</table>

*Significant difference between eye treated with ionophore and fellow eye, paired-t test, p < 0.01.
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.2

X537A together with calcium increases acetylcholine release elicited by nerve stimulation6 and A23187 causes a calcium-dependent release of catecholamines from perfused cat adrenal glands.2 X537A complexes and can transport epinephrine,5 and A23187 causes potassium release from rat parotid slices, simulating the action of epinephrine on the α-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.8 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 γ-aminobutyric acid, 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.

A generous supply of ionophore A23187 was provided by Dr. Robert L. Hamill, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, and of ionophore X537A by Dr. W. E. Scott, Hoffmann-LaRoche Inc., Nutley, New Jersey.

From the Department of Ophthalmology, Mount Sinai School of Medicine of The City University of New York, New York, N. Y. Supported in part by a research grant, EY-01661, from the National Eye Institute, Bethesda, Md. Submitted for publication April 13, 1976. Reprint requests: Dr. Steven M. Podos, Mount Sinai Medical Center, Fifth Avenue and 100th St., New York, N. Y. 10029.

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 F. 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-