with the pursuit movements of the amblyopic eye. Inaccurate pursuit responses resulted in errors of both velocity and position of the retinal image (Fig. 2). Both of these errors can be corrected slowly by pursuit movements alone, or abruptly by a combination of saccadic and pursuit movements.7 Abrupt corrections of velocity errors only occur when saccadic and pursuit movements are in the same direction.7 Inaccurate pursuit responses which are slower than their stimuli result in corrective movements for position and velocity that have the same direction, whereas responses which are faster than their stimuli result in corrective movements for position and velocity in opposite directions. Perhaps pursuit responses of the amblyopic eye were equal to or less than their corresponding stimuli (Fig. 2) to insure prompt correction for errors of position and velocity.

The directional impairment of saccadic and pursuit movements of amblyopic eyes appears to be associated with binocular suppression scotomas usually found in amblyopia.8 Most amblyopes (75 per cent) have a nasalward deviation of their amblyopic eye (esotropia),9 which is associated with suppression of the nasal hemiretina during binocular viewing conditions.10 Perhaps this nasal hemiretinal suppression scotoma constitutes a form of deprivation during early childhood development which leads to a reduction of visual acuity as well as poor resolution of both position and velocity of the retinal image. The loss of sensitivity would influence estimates of error signals for position and velocity of the retinal image and thereby interfere with the saccadic and pursuit systems of the amblyopic eye.

From the College of Optometry, Pacific University, Forest Grove, Ore. 97116. This work was supported by a Health Education and Welfare Postdoctoral Fellowship No. 2F02 EY 33987-03 sponsored by the University of California at Berkeley. Submitted for publication April 30, 1975.

Key words: amblyopia, deprivation, error signal, pursuit, saccade, strabismus, suppression.

REFERENCES


Effects of lysophosphatidyl choline and phospholipase A on the lens. EDWARD COTLIER, MARK BASKIN, AND LINDA KRESCA.

Lenses incubated for 24 or 63 hours in media containing either lysophosphatidyl choline or phospholipase A gained Na ions and water. Electrolyte imbalances and damage to lens fiber membranes occurred at lysophosphatidyl choline and phospholipase A concentrations of 7.5 µg per milliliter and 0.25 µg per milliliter, respectively. Intravitreal injection of 250 µg of lysophosphatidyl choline or 0.4 µg of phospholipase A induced posterior subcapsular cataracts which progressed to maturity only in the latter instance. This is the first demonstration of the cataractogenic effects of a naturally occurring aqueous humor phospholipid or its generating enzyme.

Common features to many experimental and human cortical cataracts are the increased concentrations of Na ions and the disruption of the fiber membranes.1-5 Na ion and water gains and fiber membrane rupture were found in cultured rabbit lenses after exposure to antibiotics or surface active agents.3 Furthermore, polymyxin B sulfate, cetyl pyridinium chloride, sodium dodecyl sulfate, or Triton X-100 can induce cataracts when injected into the vitreous cavity of the rabbit eye.4 A search for other compounds with membrane lytic activity in normal aqueous humor revealed the presence of lysophosphatidyl choline (LPC)6 which is known to lyse red blood cells in vitro.6 We report here the effects of LPC on the rabbit lens in culture and following intravitreal injection. The lens effects of phospholipase A, the enzyme which generates LPC from phosphatidyl choline (PC), was simi-
Table I. Lysophosphatidyl choline: effect on lens in culture*

<table>
<thead>
<tr>
<th></th>
<th>Tyrode's media</th>
<th>TC-199 bicarbonate-buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LPC (7.5 µg/ml.)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>5.4 ± 0.5</td>
<td>15.0 ± 0.7 P &lt; 0.001</td>
</tr>
<tr>
<td>K⁺</td>
<td>26.4 ± 0.7</td>
<td>25.0 ± 0.7 N.S.</td>
</tr>
<tr>
<td>Na⁺ + K⁺</td>
<td>32.1 ± 2.1</td>
<td>39.4 ± 2.4 P &lt; 0.01</td>
</tr>
<tr>
<td>Water increase†</td>
<td>Δ 26.2 ± 3.3</td>
<td></td>
</tr>
</tbody>
</table>

*Na⁺, K⁺, and Na⁺+K⁺ in microequivalents per lens. Mean ± standard error. Eight lenses in each group.
†Water increase in milligrams per lens in treated vs. control lenses.

Table II. Phospholipase A: effect on lens in culture*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Phospholipase A (1.0 µg/ml.)</th>
<th>Control</th>
<th>Phospholipase A (0.5 µg/ml.)</th>
<th>Control</th>
<th>Phospholipase A (0.25 µg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>4.9 ± 0.3</td>
<td>10.0 ± 0.8 p &lt; 0.001</td>
<td>5.1 ± 1.0</td>
<td>9.6 ± 1.8 p &lt; 0.05</td>
<td>4.6 ± 0.3</td>
<td>7.3 ± 0.7 p &lt; 0.01</td>
</tr>
<tr>
<td>K⁺</td>
<td>26.5 ± 2.2</td>
<td>24.3 ± 2.3 N.S.</td>
<td>25.5 ± 0.7</td>
<td>23.9 ± 1.0 N.S.</td>
<td>24.7 ± 1.1</td>
<td>21.8 ± 0.9 N.S.</td>
</tr>
<tr>
<td>Na⁺ + K⁺</td>
<td>31.3 ± 2.1</td>
<td>34.3 ± 2.2 N.S.</td>
<td>30.6 ± 0.4</td>
<td>32.5 ± 1.2 N.S.</td>
<td>28.3 ± 0.3</td>
<td>29.1 ± 1.5 N.S.</td>
</tr>
<tr>
<td>Water increase†</td>
<td>12.5 ± 0.1</td>
<td></td>
<td>10.0 ± 0.2</td>
<td></td>
<td>5.0 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Tyrode's media, 24 hours. Eight lenses in each group. Na⁺, K⁺ and Na⁺+K⁺ in microequivalents per lens. Mean ± S.E.
†Water increase in milligrams per lens in treated vs. control lenses.

cultured in vitro in Kjeldahl flasks containing 10 ml of Tyrode's media* or TC-199 bicarbonate buffer5 to which penicillin (100 units per milliliter) and streptomycin (500 µg per milliliter) were added. The contralateral lenses of each animal were incubated in a similar fashion, but with the addition of LPC (Sigma Chemical Co., St. Louis, Mo.) or phospholipase A (Calbiochem, La Jolla, Calif.). The latter was tested and found devoid of hemolytic activity. The culture media, with or without additions, was replaced every 24 hours. At the end of the culture period, water, Na⁺, and K⁺ were determined in each lens.

Lenses incubated in vitro in Tyrode's media or TC-199 bicarbonate media containing 5 µg per milliliter of LPC for 24 or 63 hours did not differ significantly from contralateral control lenses in their levels of Na⁺, K⁺, or in water content. However, when LPC in the culture media was added in concentration of 7.5 µg per milliliter, gains in Na⁺ ions and water were found in the lens after 63 hours in culture (Table I). The concentration of K⁺ ions of those LPC exposed lenses was not significantly reduced (Table I). Increased total levels of Na⁺ + K⁺ ions in LPC-exposed lenses signaled increased osmolarity according to the water flux equilibration mechanism described by Tosteson10 for red blood cells. Ten, 25, or 50 µg per milliliter of LPC in culture media induced dose-dependent increase in lens Na⁺ and water content (not shown). Phospholipase A induced similar gains of Na⁺ ions and water when added to rabbit lens culture media (Table II).

LPC and phospholipase A are water soluble and thus can be injected into the vitreous of the rabbit which contains 99.5 per cent water. The
Fig. 2. The effects of phospholipase A on the posterior cortex of the rabbit lens three hours after injection of 10 μg in the vitreous cavity: A, separation of the lens fibers by small oval and sausage-like vacuoles (v) which become confluent and enlarge into round vacuoles (V). Arrow points to free floating lens fiber membrane (x1,600). B, arrows point to ring-like membranes floating free inside large vacuoles (x26,600). C, Swiss-cheese appearance of posterior lens cortex with intact lens capsule (C), and multiple vacuolar formations (v) (x3,300).

injection into the vitreous allows testing of agents with potential cataractogenic or retinotoxic effects. Within 12 hours after intravitreal injection of LPC (250 μg or higher) or phospholipase A (0.4 μg or higher) vacuoles were noted in the posterior cortex of the lens. These long vacuoles seemed to follow the general direction of the cortical lens fibers (Fig. 1), stretching in an arcuate fashion from the posterior cortex of the lens, and in an arcuate fashion from the posterior horizontal suture, and then toward the equator within two to three days. The cataracts progressed through various morphologic stages similar to those found after intravitreal injection of polymyxin B sulfate or other surface active agents.14

The ultrastructure appearance of the lens following phospholipase A injection in the vitreous demonstrated that the lens fibers were separated by clear round vacuoles and sausage-like water spaces (Fig. 2, A). In some instances entire fiber membranes, which had adopted a ring-like appearance, were found floating within the distended extracellular spaces (Figs. 2, A and B). The capsule of the lens remained intact, whereas the water spaces between the fibers gave a typical swiss-cheese appearance to the lens stroma (Fig. 2, C).

The addition of LPC disrupts and weakens membrane hydrophobic bonding,11 thus modifying lamellar membrane structure by the formation of thermodynamically stable micellar structures.5 These conformational changes of the membranes compromise their selective permeability, perhaps by inhibiting (Na+–K+) ATPase activity, resulting in Na+ ion influx, lens fiber membrane hydration, and subsequent fiber lysis. Membrane conformational changes and compromised membrane function also occur after phospholipase A treatment of erythrocytes, after fatty acid and LPC are removed from the red cell surface in the presence of bovine serum albumin prior to hemolysis.12 The (LPC/PC + cholesterol) ratio determines whether experimental membranes assume a micellar (LPC predominates) or disc-like (PC predominates) shape in solution.12 In the aging human lens, cholesterol and sphingomyelin concentrations increase, and LPC is present in small amounts at nearly constant concentration. It is possible for membrane integrity to become compromised if the concentration ratio of membrane.
Fig. 2, B. For legend, see page 699.

Fig. 2, C. For legend, see page 699.
neutral lipid and phospholipid become abnormal. This may take place by exposing lenses to LPC or phospholipase A, or with decreased phospholipid levels as we have found in human senile cataracts (unpublished data).

The effects of LPC on Na⁺ lens levels in cultured lenses were found at concentrations of 7.5 μg per milliliter or higher. In normal rabbit aqueous humor, Varma and Reddy found 2.3 ± 1.5 μg per milliliter of LPC. We found levels of 4.3 μg per milliliter in normal rabbit aqueous humor (unpublished data). Thus, increased levels of aqueous humor LPC could lead to Na⁺ and water gains by the lens and cataracts. In rabbit plasma the concentration of LPC is 110 μg per milliliter. The breakdown of the blood-aqueous barrier insures higher LPC in aqueous. We found LPC levels of 14.2 μg per milliliter in secondary aqueous humor after the blood-aqueous barrier was disrupted by anterior chamber paracentesis (unpublished data). Following chronic intraocular inflammation, posterior subcapsular cataracts develop secondary to prolonged breakdown of the blood-aqueous or blood-vitreous barrier. Studies on LPC aqueous humor levels in patients with intraocular inflammation are needed to determine if this phospholipid represents a cataractogenic agent in uveitis.

From the Department of Ophthalmology, University of Illinois, Eye and Ear Infirmary, Chicago, Illinois. This research was supported by Grant EY-704 of the National Eye Institute. Submitted for publication March 13, 1975.

REFERENCES

Specializations of the retinochoroidal juncture. HIROHIKO MIKI, MARGARET B. BELLHORN, AND PAUL HENKIND.

The occurrence of attachment sites between the basal plasma membrane of the retinal pigment epithelium and its basement lamina have been observed in a variety of animal species, including man. Similar structures connect adjacent basal infoldings of the retinal pigment epithelium. The topographic distribution of such attachment sites and basal infoldings in rat eyes show small differences between the peripapillary, posterior, and peripheral retina. Such attachment sites may help to provide a firm connection between the retinal pigment epithelium and choroid.

It is a well-known clinical and anatomic observation that the basal surface of the retinal pigment epithelium (RP) of mammals is firmly adherent to the underlying choroid, in contrast to the more tenuous connection of the apical surface of the RPE with the adjacent outer segments of photoreceptor cells. Ultrastructural observation of the juncture of the RPE and Bruch's membrane has shown the existence of attachment sites which may help to explain this cohesion.

Materials and methods. Eyes from normal adult animals were enucleated, hemisected at the ora serrata, and immersed in 2 per cent or 4 per cent glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for three to six hours at room temperature and rinsed in buffered sucrose. Postfixation was with 1 per cent OsO₄ in the same buffer for 1 to 1.5 hours. After dehydration in ethanol and embedding in Epon 812, thin sections were cut, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 95-2 electron microscope.

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933294/ on 06/25/2017