Initial Observations of Rabbit Retinal Pigment Epithelium-Choroid-Sclera Preparations

Donald A. Frambach,*† John L. Valentine,* and John J. Weirer†

Retinal pigment epithelium (RPE)-choroid-sclera preparations from black dutch-belted rabbits were sealed in an Ussing chamber maintained at 37–39°C. Typical preparations produced a spontaneous voltage (Ve) of 12.5 mV (retina side positive) and possessed an electrical resistance (R) of 350 ohm-cm². Both of these values can be attributed to the RPE. Ouabain and amiloride diminished the Ve without affecting R. Ouabain was effective when applied to the apical but not to the basolateral side of the preparation, suggesting the presence of a Na-K ATPase on rabbit RPE apical membrane similar to that found in bullfrogs, embryonic chickens, cats and dogs. Dinitrophenol also reduced Ve. Digoxin, furosemide, bumetanide, ethacrynic acid and chlorothiazide had no apparent effect upon Ve and R. The lack of response to furosemide, bumetanide and ethacrynic acid strongly suggests that, unlike RPE from other species, rabbit RPE does not possess Na-dependent Cl transport and/or does not possess furosemide receptors on its apical membrane. Invest Ophthalmol Vis Sci 29:814–817, 1988

The transepithelial transport properties of RPE from frogs and embryonic chickens have been well studied in several laboratories.1–4 Although the transport systems observed in RPE from these two species have much in common, several major differences are apparent.4 The most obvious is that a transport system involving bicarbonate, which contributes 38% of the spontaneous trans-RPE electrical current in bullfrogs,2 is absent in embryonic chicken RPE.4 Potential species differences make it difficult to extrapolate results from bullfrog and embryonic chicken RPE to human RPE. A mammalian RPE preparation is desirable.

Initial observations of an RPE-choroid preparation from cats were published 10 years5 ago but further studies of this preparation have not been reported. RPE-choroid explants from sheep5 and cows (Miller SS, personal communication, 1986) have been studied. However, these tissues must be obtained from an abattoir, making prompt laboratory examination impractical. RPE-choroid preparations from dogs7 and monkeys8 have recently been developed. Unfortunately, these animals are expensive, prohibiting extensive study of these preparations. We have developed a method to prepare rabbit RPE-choroid-sclera explants for study in vitro. We herein describe the method of explant preparation and pharmacologic studies which show that RPE transport in rabbits differs from that seen in other species.

Materials and Methods. We adhered to the principles of the ARVO Resolution on the Use of Animals in Research. Black dutch-belted rabbits (both males and females) weighing 1.2–1.8 kg were anesthetized with xylazine 27 mg/kg IM (Miles Laboratories, Elkhart, IN) and ketamine 130 mg/kg IM (Parke-Davis, Morris Plains, NJ). Pupillary dilation was obtained with one to two drops of 1% cyclopentolate (Akorn Inc., Abita Springs, LA). The retinal pigment epithelium (RPE) was gently separated from the overlying neural retina in vivo by creating retinal detachments as previously described9 and the rabbit's eye was enucleated. A ring of acrylic plastic was glued to the sclera at the posterior pole with a cyanoacrylate cement (Histoacryl® blau, B. Braun Melsungen AG). Histoacryl has little apparent toxicity.10 The tissue surrounding the acrylic ring was cut away, and the overlying retina and vitreous simply floated off.

The RPE-choroid-sclera was sealed within an acrylic mounting chip with an aperture of 0.07 cm². The mounting chip was then sealed in a conventional Ussing chamber surrounded by a water jacket that kept its contents at 37–39°C. A schematic of the chamber and mounting chip is shown in Figure 1.

The bathing medium consisted of (in millimoles per liter): 143 Na, 3.6 K, 1.2 Ca, 2.5 Mg, 125 Cl, 23 HCO₃, 2.5 SO₄, 0.5 PO₄, 10 glucose. The solutions were equilibrated with a 5% CO₂/95% O₂ gas mixture to yield a pH of 7.4 (±0.1). The pharmacologic agents used were: ouabain, 2,4-dinitrophenol, amiloride, digoxin, furosemide, bumetanide, ethacrynic acid, chlorothiazide (Merk Sharp and Dohme, West Point, PA), and acetazolamide (Lederle Parenterals, Wayne, NJ). These agents were added to the control medium.
Fig. 1. Schematic of the apparatus. RPE/choroid/sclera separates solutions “A” and “B” which are otherwise electrically isolated and the tissue is held in a mounting chip shown in detail at the left. Voltages across the tissue are measured with a differential amplifier through Ag-AgCl electrodes connected to the chamber via agar bridges. Currents can be passed through the chamber from a stimulus isolator via bare PtIr wires. The chamber and perfusate are in 37°C water baths.

and then perfused through one or both sides of the chamber at approximately 10 ml/min. There was 4 ml of bathing media on each side of the preparation.

Results. Typical preparations produced a spontaneous voltage ($V_e$) of 12.5 mV with the apical side of the RPE positive. The electrical resistance ($R$) was typically 350 ohm-cm$^2$. When the RPE was gently scraped off Bruch’s membrane leaving the underlying choroid and sclera behind, there was no detectable $V_e$ and the R was 2.3 ohm-cm$^2$ (±0.8 SD, n = 3). Therefore, the entire $V_e$ and essentially all of the R can be attributed to the RPE. The short circuit current ($I_{sc}$) was calculated from $V_e$ and R. In typical preparations, the $V_e$, $R$, and $I_{sc}$ fell with time.

Ouabain, a potent Na-K ATPase inhibitor, (at $10^{-5}$ M) reduced $I_{sc}$ by 79% (±4% SD, n = 4). Figure 2 shows such an experiment. This effect was seen when ouabain was applied to the apical but not to the basolateral side of the RPE (n = 3). Surprisingly, digoxin, an analog of ouabain that also inhibits Na-K ATPase, had no discernible effect upon $V_e$ and R even at concentrations as high as $10^{-4}$ M (n = 3).

Dinitrophenol (DNP) reduced $I_{sc}$ by 90% (±10% SD, n = 3). These experiments appeared essentially identical to those observed with ouabain and are not illustrated.

Amiloride, an inhibitor of Na transport, had a modest effect upon $V_e$ and $I_{sc}$ and no effect upon R (n = 6) when added to both sides of the chamber. $V_e$ and $I_{sc}$ fell at a constant rate for as long as the tissue was exposed to amiloride and then recovered when amiloride was removed from the chamber. Figure 3 shows that this effect could be repeated many times on the same tissue.

Acetazolamide ($10^{-3}$ M), a carbonic anhydrase inhibitor, had no apparent effect when added to the preparation (n = 8). Similarly, the diuretics furosemide ($10^{-3}$ M, n = 5), bumetanide ($10^{-3}$ M, n = 5), and ethacrynic acid ($10^{-4}$ M, n = 3) had no apparent effect when applied to the apical and then the basolateral side of the RPE. The diuretic chlorothiazide also had no apparent effect ($10^{-3}$ M, n = 5).

Discussion. Since ouabain, a Na-K ATPase inhibitor, dramatically reduced rabbit RPE $I_{sc}$ when applied to the apical but not to the basolateral side of the tissue, we infer that, like RPE from bullfrogs, embryonic...
chickens,\(^4\) cats\(^5\) and dogs,\(^7\) rabbit RPE has a Na-K ATPase on its apical membrane. We were surprised that the ouabain analog, digoxin, had no effect upon electrical measurements. However, there are different forms of Na-K ATPase\(^\text{13}\) that, in different organs and species, have widely varying affinities for the cardiac glycosides.\(^\text{14}\)

The experiment with dinitrophenol (DNP), a metabolic poison and hence a nonspecific transport inhibitor, is consistent with reports that DNP slows bullfrog RPE transport in vitro\(^\text{1,15}\) and rabbit RPE transport in vivo.\(^\text{16}\)

Unlike its effect upon bullfrog RPE\(^1\) but similar to its effect upon embryonic chicken RPE,\(^4\) acetazolamide had no apparent effect upon \(V_e\) and \(R\) in our rabbit preparations. It has been reported that acetazolamide speeds resorption of fluid from the subretinal space of rabbits.\(^17\) Since this resorption of fluid is thought to be dependent upon active transport,\(^9\) it would seem that acetazolamide ought to affect an active transport mechanism. Our result is not inconsistent with the previous in vivo experiments for at least two reasons. First, if the transport system affected by acetazolamide is electroneutral, we would not detect either the transport mechanism or the effect of acetazolamide. Second, acetazolamide was found to be effective only if administered systemically to the rabbits,\(^17\) where it may have promoted the release of a second substance that stimulated transport or where acetazolamide itself reached the basolateral membrane of the RPE and affected transport.

In our in vitro experiments, this “second substance” may not have been present. It is also possible that the intact choroid and sclera prevented acetazolamide from reaching the RPE basolateral membrane in physiologically significant concentrations during the course of our experiments.

Furosemide, bumetanide, and ethacrynic acid are fairly sensitive and specific probes for Na-dependent Cl transport.\(^4\) Furosemide receptors have been demonstrated to be localized to embryonic chicken, bullfrog and dog RPE apical membrane.\(^1,2,4,7\) Since rabbit \(I_c\) was unaffected when the RPE apical membrane was exposed to these agents, this strongly suggests that rabbit RPE lacks Na-dependent Cl transport and/or that its apical membrane lacks furosemide receptors.

Amiloride slightly reduced \(V_e\) and \(I_c\) in rabbit RPE. This drug had no apparent effect upon embryonic chicken \(I_c\).\(^4\) To our knowledge, amiloride has not been tested with RPE from other species. Amiloride blocks Na transport processes\(^9\) and there are a number of mechanisms whereby amiloride could reduce \(I_c\). Further experiments are necessary to understand this effect.

The results of the experiments outlined in this paper indicate that rabbit RPE transport is generally

### Table 1. Responses of RPE from different species to pharmacologic agents (% change in short circuit current)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Bullfrog</th>
<th>Embryonic chicken</th>
<th>Cat</th>
<th>Dog</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinitrophenol</td>
<td>-95(^%)^(^\text{15})</td>
<td>NT</td>
<td>-88(^%)^(^3)</td>
<td>NT</td>
<td>-90(^%)</td>
</tr>
<tr>
<td>Ouabain</td>
<td>-90(^%)^(^1,2)</td>
<td>-85(^%)^(^4)</td>
<td>-MNG(^\text{1,3})</td>
<td>-84(^%)^(^7)</td>
<td>-79(^%)</td>
</tr>
<tr>
<td>apical side</td>
<td>0(^%)^(^1,2)</td>
<td>0(^%)^(^4)</td>
<td>0(^%)^(^1)</td>
<td>0(^%)^(^7)</td>
<td>0(^%)</td>
</tr>
<tr>
<td>basal side</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Digoxin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Furosemide</td>
<td>-20(^%)^(^2)</td>
<td>-67(^%)^(^4)</td>
<td>NT</td>
<td>NT</td>
<td>-38(^%)^(^7)</td>
</tr>
<tr>
<td>apical side</td>
<td>0(^%)^(^2)</td>
<td>0(^%)^(^4)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>basal side</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Bumetanide</td>
<td>-20(^%)^(^2)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>-MNG(^1)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Chlorothiazide</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>-46(^%)^(^1)</td>
<td>0(^%)^(^4)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Amiloride</td>
<td>NT</td>
<td>0(^%)^(^4)</td>
<td>NT</td>
<td>NT</td>
<td>-DE</td>
</tr>
</tbody>
</table>

NT = not tested, MNG = magnitude not given, DE = dependent upon length of exposure.
similar to that observed in frog, embryonic chicken, cat, and dog RPE. However, as summarized in Table 1, there are fundamental differences in RPE transport mechanisms between these species. Extrapolating RPE transport data among species should be done very cautiously.

**Key words:** rabbit, retinal pigment epithelium, transport

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**References**


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**Isolation of Non-Pigmented Epithelial Cells From Rabbit Ciliary Body**

Gordon L. Fain, Marianne C. Ciuffo, Margery J. Fain, and David A. Lee

We describe a new technique for the separation and isolation of nonpigmented ciliary body epithelial cells from rabbit. Excised ciliary body is incubated in a medium buffered with EGTA to a free-Ca^2+ concentration of 10^-8 M, and the nonpigmented cell layer is separated from the pigmented layer by microdissection under direct visualization. This technique yields intact cells which are greater than 99% nonpigmented. It can be used to produce preparations of nonpigmented cell plasma membrane and of viable whole cells, which may be useful for biochemistry, pharmacology, cell transport studies and tissue culture. *Invest Ophthalmol Vis Sci* 29:817–821, 1988

Aqueous humor is produced by the highly invaginated epithelium of the ciliary body. This epithelium consists of two layers of cells, a nonpigmented layer facing the posterior chamber and a heavily pigmented layer overlying the stroma of the ciliary body. Little is known about how aqueous humor is secreted across this double layer of cells or what role each layer plays in aqueous humor production. Various techniques have been used to isolate these cells, especially those from the nonpigmented layer, so that their individual properties can be investigated in vitro. One approach...