Permeability of Retinal Pigment Epithelium: Effects of Permeant Molecular Weight and Lipophilicity

Leena Pitkänen, Veli-Pekka Ranta, Hanna Moilanen, and Arto Urtti

**PURPOSE.** To determine the effects of solute molecular weight and lipophilicity on the permeability of a retinal pigment epithelium (RPE)-choroid preparation.

**METHODS.** Fresh RPE-choroid specimens from bovine eyes were placed in diffusion chambers for permeability experiments with carboxyfluorescein, fluorescein isothiocyanate (FITC)-labeled dextrans with molecular masses from 4 to 80 kDa, and β-blockers exhibiting a wide range of lipophilicity (atenolol, nadolol, pindolol, timolol, metoprolol, and betaxolol). Permeability experiments were performed both in the choroid-to-retina (inward) direction and in the retina-to-choroid (outward) direction. Carboxyfluorescein and FITC-dextrans were determined by fluorometry, and β-blockers by HPLC. The transepithelial electrical resistance and potential difference were monitored during the experiments.

**RESULTS.** Permeability of the fluorocergic FITC-dextran probes through RPE-choroid decreased significantly with the increasing size of the probe. RPE-choroid was 35 times more permeable to carboxyfluorescein (376 Da) than to FITC-dextran 80 kDa. The permeabilities of lipophilic β-blockers were up to 8 and 20 times higher than that of hydrophilic atenolol and carboxyfluorescein, respectively. The lag time of solute flux across the RPE-choroid increased with the molecular weight and lipophilicity. Compared with published data on isolated sclera, bovine RPE-choroid was 10 to 100 times less permeable to hydrophilic compounds and macromolecules. The permeability of lipophilic molecules in RPE-choroid was in the same range as in the sclera.

**CONCLUSIONS.** RPE is a major barrier and may be the rate-limiting factor in the retinal delivery of hydrophobic drugs and macromolecules through the transscleral route. For lipophilic molecules, RPE-choroid, and sclera are approximately equal barriers. (Invest Ophtalmol Vis Sci. 2005;46:641–646) DOI: 10.1167/iovs.04-1051

Problems in drug delivery to the posterior segment of the eye limit the treatment of severe ocular diseases, such as age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, and glaucoma. By using topical or systemic delivery, it is difficult to achieve therapeutic drug levels in the posterior tissues of the eye with reasonable doses. Topical drug delivery to the posterior segment is considered to be insufficient due to the following reasons: rapid drainage of eyedrops, anterior membrane barriers (cornea, conjunctiva, and sclera), systemic absorption via conjunctival vessels, and aqueous humor outflow.1,2 Ocular delivery of intravenous drugs is limited by a blood-retinal barrier that is composed of retinal capillaries and the retinal pigment epithelium (RPE).3,4 For these reasons, massive intravenous doses, intravitreal implants, and intraocular injections are sometimes used to treat posterior segment diseases. Invasive ocular injections may cause side effects, such as infections and retinal detachment,5 and safer methods of delivery are therefore needed.

Transscleral drug delivery after subconjunctival, sub-Tenon’s, or retrobulbar application may be a feasible way to deliver drugs, bioactive proteins (e.g., growth factors or antiangiogenic agents), and gene medicines to the retina.2,5 In those cases, the drug should permeate the sclera, choroid, and RPE. Scleral permeability declines with the increasing size of the molecule, but the sclera is still quite permeable, even to high-molecular-mass compounds.6,7 Solute lipophilicity, however, does not seem to affect scleral permeability markedly.8 The next barriers, the choroid and especially Bruch’s membrane, may be significant permeation barriers for some macro-molecules.9 The RPE is considered to be a tight barrier because of its tight intercellular junctions.10,11 Surprisingly, systematic data on the permeability characteristics of the RPE are not available.

The RPE also restricts the ocular absorption of drugs after systemic administration.2,5,4 Drugs in the bloodstream rapidly equilibrate with the extravascular space of the choroid, because the choriocapillaris is fenestrated. RPE limits the permeation of drugs from the choroid to the retina. Therefore, it forms the outer part of the blood-retinal barrier.

The RPE is a monolayer of highly specialized cuboidal cells located between the neural retina and the choroid.12 It has essential biochemical functions in maintaining the visual system, including phagocytosis of photoreceptor outer segments, transport of substances between photoreceptors and the choriocapillaris, and uptake and conversion of the retinooids that are needed in the visual cycle. The tight junctions of the RPE restrict the intercellular permeation of molecules efficiently. In a study of monkeys, the permeation of horseradish peroxidase (44 kDa) stopped at the tight junctions of the RPE.10,13 In addition, binding of drugs to the tissue proteins or melanin pigment, active transport processes, and metabolism may affect RPE permeability.10,14 Current literature does not provide adequate information regarding RPE permeability as such or in comparison to other ocular membranes. Therefore, the purpose of this study was to provide systematic data about the permeability of RPE-choroid as a function of solute size and lipophilicity. The permeation of molecules of various sizes and lipophilicities was determined in isolated bovine RPE-choroid, in both the choroid-to-retina (inward) and the retina-to-choroid (outward) directions. Carboxyfluorescein (376 Da), five FITC-dextrans (4–80 kDa), and six β-blockers were used as probes. FITC-dextrans are nontoxic polymers that have been used in vitro permeation studies of ocular membranes1,7 and in vivo experiments11,15—for example, after injection into the subretinal or vitreal space. β-Blockers are optimal probes to study the effect of lipophilicity on membrane permeability, because they have almost equal...
molecular size (molecular masses typically between 250 and 320 Da) and ionization constant (bases with pK_a ~9), but their lipophilicity varies over a wide range.16-17

**MATERIALS AND METHODS**

**Tissue Preparation**

The experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Fresh bovine eyes were obtained from a local abattoir and kept at approximately 9°C during transport to the laboratory. They were cleaned of extracellular material and dipped in 0.9% NaCl solution. The eyes were opened circumferentially approximately 8 mm behind the limbus and the anterior tissues and the vitreous was separated gently from the neural retina. A circular piece with a diameter of 20 mm was cut with a punch from the pigmented part of the eyecup. The sclera was detached with forceps, and the retina-choroid block was placed in physiologic saline (BSS Plus; Alcon, Fort Worth, TX). The neural retina was separated gently, and the RPE-choroid specimen was moved carefully onto a piece of nylon mesh ( pores, 1 × 1 mm), with the choroid part facing the nylon mesh.

**In Vitro Diffusion Apparatus**

The tissue block with the nylon mesh was placed in a vertical diffusion chamber (Costar, Cambridge, MA) equipped with silicone adapters with a circular aperture of 9.5 mm (exposed area, 0.709 cm²). Vacuum grease was used to seal the margins of the tissue to the adapters. In the experiments with fluorescent probes, 5 mL of prewarmed diffusion medium (described later) was pipetted onto both sides of the tissues. After an equilibration period of at least 5 minutes, a fluorescent probe (described later) was added to either the choroidal or retinal side of the tissue. In β-blocker studies, the experiment was started without an equilibrium period by pipetting 5 mL of diffusion medium to the receptor side and an equal volume of test solution (described later) to the donor side. The chamber was maintained at 37°C by using a heating block (Costar) and a circulating water bath (M3; Lauda, Königsborn, Germany). Bubbling with low oxygen gas (5% CO₂, 10% O₂, 85% N₂) provided oxygen to the tissue and mixed the donor and receptor solutions during the experiment. In addition, CO₂ maintained the pH at 7.4 to 7.5 during the experiment.

**Diffusion Medium and Test Solutions**

A saline solution (BSS Plus; Alcon), containing glutathione, glucose, bicarbonate, and electrolytes, was used as the diffusion medium, because the combination of glutathione and low oxygen gas increases the longevity of RPE-choroid preparations.19 In the experiments with fluorescent probes, 1 mL of the diffusion medium was removed from either the choroidal or retinal side of the tissue and replaced with an equal volume of the probe-containing medium to start the experiment. The initial concentration of 6-carboxyfluorescein (Sigma-Aldrich, St. Louis, MO) in the donor chamber was 0.0376 mg/mL (100 μM). The initial donor concentrations of FITC-dextran with mean molecular masses of 4.4, 9, 23, 58.2, or 77.0 kDa (Sigma-Aldrich) were 4, 6, 6, 3, 4, 9, 23, 58.2, or 77.0 kDa (Sigma-Aldrich) were 4, 6, 6, 8, or 8 mg/mL (909, 645, 283, 209, or 104 μM), respectively. To maintain a constant pH during the β-blocker studies, HEPES (Sigma-Aldrich) was dissolved in the balanced saline solution at a concentration of 10 mM, and the pH of the solution was adjusted to 7.4. The test solution was a mixture of atenolol, nadolol, pindolol, metoprolol (all from Sigma-Aldrich), betaxolol (donated by Alcon, Fort Worth, TX), and timolol (donated by Merck Sharp & Dohme Research Laboratory, Rahway, NJ) in the saline solution with 10 mM HEPES (pH adjusted to 7.4). The concentration of metoprolol and timolol in the test solution was 100 μM, and the concentration of the other β-blockers was 400 μM. The solutions were protected from light by covering the whole diffusion apparatus with foil.

**Bioelectric Measurements**

The diffusion chamber was equipped with electrode caps (Costar), and the transepithelial electrical potential difference (TEP) and transepithelial resistance (TEER) were monitored at approximately 30-minute intervals with glass barrel microereference Ag/AgCl electrodes (Precision Instrument Design, Tahoe City, CA) and a voltage-current clamp (VCC MC 6; Physiologic Instruments, San Diego, CA). The measurements were conducted under open-circuit conditions. Current pulses of ±0.6 μA were imposed for 2 seconds across the tissue, and the change in TEP was monitored to calculate TEER. If any of the TEERs was below 70 Ω cm², the experiment was discarded. The TEER of each tissue specimen was defined as the average of all the TEERs starting from 20 to 50 minutes and ending at 210 to 240 minutes. In most cases, the individual TEERs at different times remained within the average ±20%. For TEP measurements, the asymmetry between the voltage measuring electrodes was compensated for, both before and after the experiment, to obtain accurate TEPs (the vitreal side of RPE is positive) at 20 to 30 minutes and at 210 to 240 minutes.

**Sample Collection and Analysis**

Samples of 200 μL (carboxyfluorescein and FITC-dextran) or 600 μL (β-blockers) were taken from the receptor solution at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes and replaced with pure diffusion medium. The experiments with fluorescent probes used one sample of 40 μL was taken from the donor solution at 10 minutes for the determination of the exact donor concentration. In all experiments, donor samples were also taken at 240 minutes.

Carboxyfluorescein and β-blocker samples were stored at −20°C until analyzed. FITC-dextrans were analyzed immediately after the experiment, because there was some evidence that the freezing and thawing cycle reduced the fluorescence of the most dilute samples (data not shown). Carboxyfluorescein and FITC-dextran samples were protected from light at all times before fluorescence measurements. Carboxyfluorescein and FITC-dextran were determined with a 96-well fluorescence plate reader (FL 500; Bio-Tek Instruments, Burlington, VT) with 485 nm excitation and 530 nm emission filter. Standard curves of fluorescence versus concentration were obtained by serial dilution of the fluorescent compounds in diffusion medium. Concentrations in samples were determined by linear regression analysis within the linear portion of the standard curve. The β-blockers were determined using gradient HPLC with combined ultraviolet (UV) and fluorescence detection. The HPLC system consisted of a programmable solvent module (System Gold 168; Beckman Instruments, Fullerton, CA) with a diode array UV detector and an autosampler (model 507c; Beckman Instruments) with a 50-μL sample loop. A column temperature controller (Meta Therm; MetaChem Technologies, Torrance, CA) was used with the HPLC system. A fluorescence detector (1046A; Hewlett-Packard, Waldbronn, Germany) was used, together with UV detection, and signals from the two detectors were collected by the accompanying software (32 Karat ver. 3.0; Hewlett-Packard). The chromatographic conditions and calibration procedures have been described elsewhere.19

**Calculation of Apparent Permeability Coefficient and Permeation Lag Time**

The diffusion of the solutes across RPE-choroid was characterized by calculating the apparent permeability coefficient (P_app, cm/sec) as P_app = flux/(Savg×C0), where flux is the slope of the linear portion of the permeability curve (cumulative amount appearing in the receptor solution versus time) for each probe molecule (in nanomoles per second), Savg is the exposed surface area of the RPE-choroid tissue (0.709 cm²), and C0 is the initial concentration of the probe molecule in the donor solution (in nanomoles per cubic centimeter). The permeation lag time (minutes) for each probe was determined by extrapolating the linear portion of the permeability curve and determining its intercept on the time axis.

**Statistical Analysis**

Kruskal-Wallis analysis was used to compare multiple experimental groups. When the difference was significant (P < 0.05), multiple comparisons versus a control group were performed with the Dunn
TABLE 1. The Permeability of RPE-Choroid to Fluorescent Probes of Various Molecular Weights

<table>
<thead>
<tr>
<th>Probe</th>
<th>Molecular Weight (Da)</th>
<th>Molecular Radius* (nm)</th>
<th>Diffusion Direction†</th>
<th>Permeability Coefficient (×10⁻⁷ cm/sec)</th>
<th>Permeation Lag Time (min)</th>
<th>Transepithelial Electrical Resistance (Ω × cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxyfluorescein</td>
<td>376</td>
<td>0.5</td>
<td>Inward</td>
<td>9.56 ± 3.87</td>
<td>29.7 ± 8.8</td>
<td>120 ± 13</td>
</tr>
<tr>
<td>FITC-dextran 4 kDa</td>
<td>4,400</td>
<td>1.3</td>
<td>Inward</td>
<td>23.3 ± 10.6‡</td>
<td>22.4 ± 10.8</td>
<td>114 ± 32</td>
</tr>
<tr>
<td>FITC-dextran 10 kDa</td>
<td>9,300</td>
<td>2.2</td>
<td>Inward</td>
<td>2.56 ± 1.56</td>
<td>58.3 ± 13.4</td>
<td>134 ± 41</td>
</tr>
<tr>
<td>FITC-dextran 20 kDa</td>
<td>21,200</td>
<td>3.2</td>
<td>Inward</td>
<td>2.14 ± 1.02</td>
<td>78.6 ± 35.7§</td>
<td>108 ± 21</td>
</tr>
<tr>
<td>FITC-dextran 40 kDa</td>
<td>38,200</td>
<td>4.5</td>
<td>Inward</td>
<td>1.34 ± 1.80§</td>
<td>67.5 ± 41.3</td>
<td>106 ± 15</td>
</tr>
<tr>
<td>FITC-dextran 80 kDa</td>
<td>77,000</td>
<td>6.4</td>
<td>Inward</td>
<td>0.46 ± 0.29§</td>
<td>77.5 ± 14.9§</td>
<td>106 ± 11</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD, n = 5–8.

* Molecular (Stokes-Einstein) radii were taken from the literature.†
‡ Significantly different from carboxyfluorescein in the inward direction (P < 0.01).
§ Significantly different from carboxyfluorescein in the inward direction (P < 0.05).

RESULTS

Tissue Viability

The viability of isolated RPE-choroid specimens was monitored by determining their bioelectric properties. In the experiments with fluorescent probes, TEER was 115 ± 23 and 124 ± 29 Ω × cm² (mean ± SD, n = 35–40) at 20 and 210 to 240 minutes, respectively. In β-blocker studies, TEER was 125 ± 35 and 125 ± 32 Ω × cm² (n = 14) at 30 and 210 to 240 minutes, respectively. The average TEER in different experiments was shown in Tables 1, 2; there were no significant differences between these values. In the experiments with fluorescent probes, TEP was 6.9 ± 2.3 and 5.5 ± 2.5 mV at 20 and 210 to 240 minutes (n = 35–40), respectively. In β-blocker studies, TEP was 6.4 ± 2.9 and 3.4 ± 1.5 mV (n = 14) at 30 and 210 to 240 minutes, respectively. There were no significant differences between the initial and final TEP in the different experiments.

Permeability of Carboxyfluorescein and FITC-Dextran

The inward (choroid-to-retina) permeability of carboxyfluorescein (100 μM) was 2.4 times lower than in the opposite direction, and the difference was significant (Table 1). There was no directionality in the permeation of 10-kDa FITC-dextran (645 μM). The inward permeability of fluorescent probes decreased with the increasing size of the molecule (Table 1, Fig. 1). There was a 35-fold difference in permeability between carboxyfluorescein and 80-kDa FITC-dextran. The permeability declined roughly exponentially with increasing radius (Fig. 2). The permeation lag time increased clearly with the molecular mass, between 30 and 100 minutes (Table 1, Fig. 1).

Permeability of β-Blockers

The inward permeability of the most hydrophilic β-blockers atenolol and nadolol, was two times higher than that of carboxyfluorescein (Tables 1, 2). The inward permeability coefficients of the most lipophilic β-blockers metoprolol, timolol, and betaxolol were seven to eight times higher than atenolol (Table 2, Fig. 3). Atenolol and nadolol permeated at the same rate in both directions, whereas the more lipophilic β-blockers permeated at a faster rate in the inward direction (Table 2, Fig.

TABLE 2. The Permeability of RPE-Choroid to β-Blockers of Various Lipophilicities

<table>
<thead>
<tr>
<th>β-Blocker</th>
<th>Log D*</th>
<th>Inward Diffusion (×10⁻⁶ cm/sec)</th>
<th>Outward Diffusion (×10⁻⁶ cm/sec)</th>
<th>Permeation Lag Time (min)</th>
<th>Inward Diffusion (min)</th>
<th>Outward Diffusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>−1.77</td>
<td>2.21 ± 0.50</td>
<td>2.00 ± 0.47</td>
<td>38.7 ± 10.5</td>
<td>32.6 ± 14.5</td>
<td></td>
</tr>
<tr>
<td>Nadolol</td>
<td>−1.06</td>
<td>2.24 ± 0.54</td>
<td>2.03 ± 0.46</td>
<td>41.5 ± 11.3</td>
<td>35.8 ± 15.8</td>
<td></td>
</tr>
<tr>
<td>Pindolol</td>
<td>−0.07</td>
<td>5.62 ± 1.87</td>
<td>3.48 ± 1.69</td>
<td>99.7 ± 18.1†</td>
<td>97.7 ± 11.8†</td>
<td></td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.05</td>
<td>18.8 ± 4.34§</td>
<td>10.6 ± 3.19‡</td>
<td>96.5 ± 16.0†</td>
<td>90.4 ± 18.7†</td>
<td></td>
</tr>
<tr>
<td>Timolol</td>
<td>0.09</td>
<td>14.5 ± 3.48§</td>
<td>8.41 ± 2.67‡</td>
<td>96.7 ± 16.2‡</td>
<td>99.0 ± 18.8†</td>
<td></td>
</tr>
<tr>
<td>Betaxolol</td>
<td>1.59</td>
<td>16.7 ± 4.48‡</td>
<td>10.3 ± 3.65‡</td>
<td>107 ± 12.0†</td>
<td>67.5 ± 16.3‡</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD, n = 7. Inward diffusion is the choroid-retina direction and outward diffusion is the retina-choroid direction. β-Blockers were used as a mixture, and TEER of tissues in inward experiments (115 ± 32 Ω × cm²) was not significantly different from that in outward experiments (126 ± 35 Ω × cm²).

* Logarithm of the apparent distribution coefficient between octanol and aqueous buffer solution (pH 7.4); calculated from data in the literature.†
‡ Significantly different from atenolol (P < 0.05).
§ Significantly different from inward diffusion (P < 0.01).
¶ Significantly different from inward diffusion (P < 0.05).
DISCUSSION

Transscleral drug delivery after pericocular drug application or gene transfer to extracellular cells to produce therapeutic proteins are interesting possibilities for treatment of retinal diseases. For the development of such treatments, it is important to know the permeability of the RPE, among other barriers. The permeability of the RPE also affects the permeation of drugs from the choroidal circulation to neural retina after systemic administration and the elimination of drugs from the vitreous after intravitreal injection. Permeation rates and transport mechanisms of only sparse and individual drug-like molecules in RPE are known. However, systematic data on the permeate properties of molecules as a function of size and lipophilicity is needed to predict the permeabilities of new compounds in RPE and to compare the barrier properties of RPE with other ocular membranes. The purpose of this study was to produce such data.

RPE is a delicate tissue, and it has to be isolated carefully to preserve its viability. In the present study, the bioelectric properties (TEER and TEP) of the isolated bovine RPE-choroid were similar to those in earlier studies. In addition, the permeability of carboxyfluorescein (100 μM) in the outward direction was 2.4 times higher than in the opposite direction (Table 1), suggesting active transport similar to that observed in isolated rabbit and dog RPE-choroid. The inward permeability of carboxyfluorescein in bovine RPE-choroid (0.956 ± 0.387 × 10⁻⁶ cm/s) was close to that in rabbit and dog tissues (0.5–2 × 10⁻⁶ cm/sec).

The permeability of the hydrophilic fluorescent probes carboxyfluorescein and FITC-dextran through the bovine RPE-choroid declined roughly exponentially with increasing molecular radius (Fig. 2). The major barrier was most likely the tight junctions in the RPE. It has been shown that the choroidal electrical resistance (~9 Ω × cm²), probably including Bruch’s membrane, is <10% of the total resistance of isolated bovine RPE-choroid (110–150 Ω × cm²). Recently, the permeability of 4- and 40-kDa FITC-dextran through the human Bruch’s membrane-choroid was determined to be in the range of 160 to 400 × 10⁻⁷ and 8 to 100 × 10⁻⁷ cm/sec, respectively, with a significant age-related decline in permeability (Hussain AA, unpublished data, 2004). These values are much higher than the permeabilities through the bovine RPE-choroid (Table 1), indicating that RPE is the major barrier in the isolated tissue of the experiments.

FITC-dextran are taken up into some cells by fluid-phase endocytosis. However, the process does not result in dextran transport across the tight epithelium. This process was also seen in the present study, as the permeability of 10-kDa FITC-dextran was similar in both directions (Table 1). Active transcytosis would result in asymmetry in the permeability rates.

It is interesting to compare the barrier properties of RPE-choroid with those of the sclera. Scleral permeability declines with the increasing size of the solutes, as demonstrated in human, rabbit, and bovine sclera. The permeabilities of fluorescent probes through bovine RPE-choroid, human sclera, and rabbit sclera are compared in Figure 5. The bovine RPE-choroid is approximately 10 to 100 times less permeable than human and rabbit sclera. Also, the permeability of carboxyfluorescein through human and rabbit sclera (12–13 × 10⁻⁶ cm/sec) is approximately 10 times higher than the inward permeability of carboxyfluorescein through bovine RPE-choroid in the present study (Table 1). This implies that RPE may be the rate-limiting permeation barrier in the retinal drug delivery of hydrophilic molecules and macromolecules, such as proteins and oligonucleotides, via the transscleral route.

The high permeability of sclera is due to its structure, which is comparable to corneal stroma. The molecules are expected to diffuse through the interfibrillar aqueous media of the gel-like proteoglycans. Scleral permeability is probably inversely related to its thickness because of its fairly uniform structure. The thickness of human sclera varies generally between 0.4

FIGURE 1. Permeation of fluorescent probes across the RPE-choroid in an inward (choroid-to-retina) direction (mean ± SEM, n = 5–8). Probes: carboxyfluorescein (■) and FITC-dextran with molecular masses of 4 kDa (□), 10 kDa (▲), 20 kDa (△), 40 kDa (●), and 80 kDa (○).

4). The TEER of the tissues used in the inward experiments was slightly lower than that in the outward experiments, but the difference was not significant (Table 2). The lag times of atenolol and nadolol were fairly similar to carboxyfluorescein, ~40 minutes, whereas the lag times of the more lipophilic β-blockers were generally ~100 minutes (Table 2). The lag times of β-blockers were similar in both directions, except for betaxolol (Table 2).

The high permeability of sclera is due to its structure, which is comparable to corneal stroma. The molecules are expected to diffuse through the interfibrillar aqueous media of the gel-like proteoglycans. Scleral permeability is probably inversely related to its thickness because of its fairly uniform structure. The thickness of human sclera varies generally between 0.4
and 1.0 mm in different anatomic locations, but is only 0.1 to 0.25 mm at the equator in a significant number of eyes. The thickness of human sclera was not reported in the study used in Figure 5, but even if these values are increased fivefold, the RPE remains the major barrier, especially for macromolecules.

In the present study, the effect of solute lipophilicity on the permeation across the RPE-choroid was determined by using \( \beta \)-blockers as test compounds (Fig. 3). The permeability of the most hydrophilic \( \beta \)-blockers atenolol and nadolol was close to that of carboxyfluorescein (Tables 1, 2), and all these compounds are expected to permeate mainly through the tight junctions of RPE (paracellular route). The permeability of the more lipophilic \( \beta \)-blockers was significantly higher, and these compounds permeate the cell membranes of the RPE (transcellular route). The range between the highest and the lowest permeabilities (metoprolol versus atenolol) was eight- and fivefold in the inward and outward directions, respectively. Scleral permeability is known to be less sensitive to solute lipophilicity. In human and rabbit sclera, the permeability of both hydrophilic and lipophilic low-molecular-weight drugs is typically in the range of 10 to 40 \( \times 10^{-6} \) cm/sec. When these scleral permeabilities are compared with the permeability of bovine RPE-choroid (Fig. 4), the RPE-choroid is the major barrier to the hydrophilic drugs, whereas both sclera and RPE-choroid are significant barriers to lipophilic drugs.

The asymmetry in the permeability of lipophilic \( \beta \)-blockers in different directions may indicate the contribution of active transporters or efflux pumps. The P-glycoprotein efflux pump has been found in both apical and basolateral cell membranes of human RPE, and many \( \beta \)-blockers are substrates or inhibitors of P-glycoprotein. However, in the present study, the asymmetry in the permeability of lipophilic \( \beta \)-blockers was fairly small. At the moment, the potential role of efflux pumps is unknown and would require thorough mechanistic studies.

The permeation lag time through the RPE-choroid increased with the size and lipophilicity of the compounds (Tables 1, 2). For fluorescent probes, the range in the lag times extended from 30 minutes to 100 minutes (Table 1). The effect of molecular weight on the lag time was expected, since the...
diffusion coefficient of the molecule in the membrane decreases with increasing molecular weight, and the lag time is generally inversely proportional to the diffusion coefficient.\textsuperscript{34} The lag times of fluorescent probes in bovine RPE-choroid were longer than in human and rabbit sclera, where the steady state permeability was achieved in 15 to 30 minutes, even for the largest FITC-dextrans.\textsuperscript{1,7} This is also expected based on the higher diffusivity in sclera.

The lag times of the lipophilic $\beta$-blockers were longer than those of the hydrophilic $\beta$-blockers (Table 2). This may be due to the binding with melanin pigment in the RPE cells and in the melanocytes of the outer choroid. Lipophilic $\beta$-blockers are known to bind with melanin.\textsuperscript{35–37} In diffusion experiments, melanin-binding increases the lag time, since the steady state permeability was achieved in 15 to 30 minutes, even for melanin-bound drug has been achieved. For this reason, the permeabilities of drugs in porcine RPE-choroid, determined using only a 30-minute incubation,\textsuperscript{25} may be lower than true steady state permeabilities. The long lag times of permeation emphasize the need for continuous or prolonged drug delivery, as the permeability is low in the beginning.

In summary, the present data give background to understanding drug permeation to the retina. It seems that the RPE is a tighter barrier than the sclera for hydrophilic and large molecules, and, therefore, scleral permeability alone is not sufficient to predict the drug delivery rate to the retina. For lipophilic drugs, RPE-choroid and sclera are equally important barriers. Although this study reveals the relative importance of RPE and sclera as barriers, the role of choroidal blood flow in transscleral drug delivery remains open. The blood flow may eliminate some drugs during permeation. Systematic data on the permeability of RPE are useful in evaluation of the retinal entry of drugs after transscleral and systemic drug delivery. Such data are essential in building pharmacokinetic simulation models to obtain quantitative estimates of drug delivery rate and to finally and truly understand the relative roles of ocular barriers in drug delivery.

References