A Na/H Exchange Mechanism in Apical Membrane Vesicles of the Retinal Pigment Epithelium
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The retinal pigment epithelium (RPE) interposed between the vascular system of the choroid and the neural retina performs a variety of functions essential for vision. In order to further elucidate the transport functions of the RPE, apical membranes were isolated from the RPE of the dogfish (Squalus acanthias) by differential precipitation with calcium. Na-K-ATPase, an apical marker enzyme in this tissue, was enriched 15-fold in the final membrane fraction. About 50% of the membranes form right-side-out vesicles in which the membrane has retained its in vivo orientation. Sodium uptake into these vesicles as determined by a rapid filtration method was stimulated 37% by the presence of a proton gradient across the membrane (pH$_{o}$ = 6.1, pH$_{l}$ = 8.1). The stimulation was also observed in membrane vesicles “short-circuited” with valinomycin and K. The pH gradient-dependent sodium uptake but not the uptake in the absence of a pH gradient was completely inhibited by 5 X 10$^{-4}$ M amiloride, and 56% inhibition was found at 10$^{-5}$ M amiloride. The uptake of $^{22}$Na was also strongly decreased in the presence of nonradioactively labelled sodium and lithium; potassium was without effect. pH gradient dependence, amiloride sensitivity, saturability and cation specificity of the sodium flux indicate the presence of a Na/H exchanger in the apical membrane of the retinal pigment epithelium. The presence of the Na/H exchange process might have important implications for the control of pH in the subretinal space, optimum intracellular pH of the RPE and the triggering of other functions of the RPE. Invest Ophthalmol Vis Sci 30:2332–2340, 1989

The retinal pigment epithelium interposed between the vascular system of the choroid and the neural retina performs diverse functions that have elicited a renewed interest in the cellular physiology and the cell biology of this epithelial layer. Its functions fall in several categories: (1) It performs as a barrier and transport organ for solutes and water between the blood and the subretinal space. (2) It has been demonstrated to have “glial-like” properties, especially in the rapid accumulation of K ions liberated into the subretinal space during retinal illumination. This K accumulation explains the origin of the c wave of the electroretinogram. (3) It controls the access of precursors of rhodopsin from the blood and accumulates products of the visual pigment by means of specific binding proteins in the membranes of the cell. (4) The retinal pigment epithelium maintains the length of photoreceptors by phagocytosis and digestion of the distal tip of the photoreceptors.

In this work we are concerned with the solute movements occurring in this epithelial cell. Active sodium transport from blood to the retinal side has been demonstrated in isolated pigment epithelium of amphibians,$^{1-3}$ as well as active chloride transport across the epithelium from the apical to the basolateral side. A strong effect of bicarbonate ions on electrical current has been well documented in several instances,$^{1,3}$ indicating a dependence of sodium fluxes either on pH or on the actual presence of bicarbonate.

In chick embryo retinal pigment epithelium, furosemide-sensitive net retina-to-choroid sodium chloride flux was observed in flux studies,$^{5}$ and on the basis of electrical measurements a pH-sensitive conductive pathway for K has been described.$^{6}$

From such studies the following transport elements have been proposed to be present in the plasma membranes. Based on the sidedness of the inhibitory effect of ouabain and histochemical and immunochemical studies, the luminal membrane is supposed to contain a high concentration of Na-K-ATPase. This membrane is also supposed to contain a sodium chloride cotransporter (operating either with or with-
out potassium) and a sodium bicarbonate cotransporter.\textsuperscript{7–12}

In order to define directly the transport systems present in the luminal membrane of the retinal pigment epithelium, we attempted in this study to isolate luminal plasma membrane vesicles from this epithelium and to study their transport properties with regard to sodium uptake. The results presented in this paper demonstrate that a membrane vesicle fraction highly enriched in Na-K-ATPase can be obtained from the retinal pigment epithelium of the spiny dogfish \textit{(Squalus acanthias)} which, based on \textsuperscript{22}Na uptake studies, contains a sodium/proton exchanger. These findings strongly support the view that retinal pigment epithelial cells—as also concluded from studies on cultured pigment epithelial cells\textsuperscript{13–15}—compensate intracellular acidification via a sodium-dependent mechanism. The location of the exchanger in the luminal membrane could, in addition, have consequences for the regulation of sodium concentration and the pH in the subretinal space. The composition of ions in this space is essential for optimum function of the retina as well as for retinal attachment.

Part of the data have been presented in preliminary form.\textsuperscript{16,17}

\section*{Materials and Methods}

\subsection*{Isolation of Plasma Membranes}

Dogfish \textit{(Squalus acanthias)} of either sex were taken by gill nets and by hook and line from Frenchmen Bay, Maine and kept in a marine live car until used, usually within 3 days of capture. Dogfish were killed by segmental transection of the spinal cord. Eyes were enucleated and placed into shark Ringer's solution,\textsuperscript{4} and kept in the dark for 30 min in order to allow the microvilli of the RPE to retract from the retina. The eye cups were obtained, the retinas removed and the RPE–choroid separated from the sclera. Tissue from several fish were combined and stored at \(-70^\circ\text{C}\) for no more than 3 days. For a typical membrane isolation about 6.0 g of tissue was employed. The frozen tissue was minced with scissors and homogenized in a Waring blender for 60 sec at full speed in 35 ml of buffer (10 mM mannitol, 2 mM Tris Cl, pH adjusted with NaOH to 7.1). The apical membranes were then enriched by a modification of the differential precipitation method described by Booth and Kenny.\textsuperscript{18} In brief, a 1 M CaCl\textsubscript{2} solution was added to the homogenate to achieve a final concentration of 30 mM Ca. The suspension was kept on ice for 15 min under occasional stirring and then centrifuged for 15 min at 2500 \(g\) at 4\(^\circ\text{C}\). The first black sediment with the melanin granules was discarded and the supernatant was filtered through cheesecloth in order to remove residual melanin granules. The centrifugation was repeated twice and all sediments were discarded. The last supernatant was then centrifuged for 45 min at 46,000 \(g\); the final pellet was composed of a dark central spot and a white layer of membranes. The white layer was carefully removed, suspended in a small volume of vesicle buffer (300 \(\mu\)l) and homogenized by repeated suction through a 25-gauge needle.

\subsection*{Determination of Enzymes and Protein}

\textbf{Enzyme determination:} The activity of Na-K-ATPase (E.C. 3.6.1.3) was routinely determined at 37\(^\circ\text{C}\) in 50 mM imidazole buffer (pH 7.6 adjusted with HCl) in the presence of 6 mM MgSO\textsubscript{4}, 3 mM Tris-ATP, 0.2 mM EDTA, 100 mM NaCl, and 20 mM KCl. The difference in enzyme activity observed in the absence or in the presence of 2 mM ouabain was defined as Na-K-ATPase.\textsuperscript{19} The samples were freeze-dried before enzyme determination since this procedure gave the highest Na-K-ATPase activities. Attempts to activate the enzyme by detergents such as deoxycholate failed. In experiments related to the question of the sidedness of the membrane vesicles, separate determinations in the presence of Mg alone, Mg, Na and K, and in the presence of Mg, Na, K and ouabain were performed on freshly isolated and on freeze-dried samples. The enzyme reaction was terminated by addition of an identical volume of ice-cold trichloroacetic acid (TCA). The amount of inorganic phosphate liberated was determined by a modified colorimetric assay according to Fiske-Subbarow.\textsuperscript{20}

The activity of 5' nucleotidase (3.1.3.5) was estimated by using a reaction mixture containing 100 mM Tris-Cl, adjusted to pH 7.6 with NaOH, 200 mM KCl, 100 mM MgCl\textsubscript{2}, 100 mM Na-K-tartrate, and 5 mM AMP as substrate.\textsuperscript{21} The reaction was terminated by the addition of 10\% TCA and the P\textsubscript{i} released was determined as described above.

The activity of acid phosphatase and NADH-dehydrogenase was determined as previously described.\textsuperscript{22} In order to obtain reliable determinations in the cell homogenate, the samples were centrifuged for 30 sec at 13,000 \(g\) after termination of the enzyme reaction to remove the melanin and guanin granules.

\textbf{Protein determination:} The protein concentration of the fractions was measured after precipitation of the protein by 10\% TCA in the cold and solubilization of the precipitate in 1 N NaOH according to Lowry using bovine serum albumin as standard.\textsuperscript{23}
Electron Microscopy

The membrane fractions were fixed in suspension with 2.5% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.4). After osmication (1% OsO₄) the material was stained with 2% uranyl acetate, dehydrated, and embedded in Spurr's low viscosity resin. For freeze-fracture experiments, vesicles were fixed with 2.5% glutaraldehyde and infiltrated with glycerol up to 40%. Fracturing was carried out in a Leybold (EPA 100, Cologne, West Germany) freeze-fracture apparatus. The platinum carbon replicas were examined in a Phillips 300 electron microscope.²⁴

Transport Experiments

After isolation the vesicles were suspended in a small volume of vesicle buffer (100 mM mannitol, 1 mM CaSO₄, and 50 mM MES, adjusted to pH 7.1 with Tris) and used immediately for the transport studies. In order to adjust the intravesicular pH (pH₄) the vesicles were then incubated for 2 hr at 15°C either in 100 mM mannitol, 1 mM CaSO₄, and 50 mM MES, adjusted with Tris to pH 6.1, or in 100 mM mannitol, 1 mM CaSO₄, 50 mM HEPES, adjusted with Tris to pH 8.1. In experiments where amiloride was used the preincubation medium contained in addition 5 x 10⁻⁴ M amiloride. When the effect of valinomycin was investigated the preincubation medium contained in addition 50 mM K gluconate and either valinomycin (5 μg dissolved in ethanol) or the equivalent amount of ethanol.

Sodium uptake was measured by a rapid filtration technique in a medium containing 100 mM mannitol, 1 mM CaSO₄, 50 mM HEPES, adjusted to pH 8.1 with Tris, 0.5 mM Na₂SO₄, and 20 μCl⁻²⁵Na. In the valinomycin experiments the medium contained in addition 50 mM K gluconate. Uptake was measured at 15°C. In brief, 20 μl of preincubated vesicles were added to 130 μl transport medium, and uptake was terminated after various times of incubation by diluting 20 μl of the sample into 1 ml of ice-cold stop solution (100 mM mannitol, 150 mM MgCl₂, 1 mM CaSO₄, and 20 mM HEPES, adjusted to pH 8.1 with Tris). After dilution the membrane suspension was rapidly filtered through a filter kept under suction (Millipore HAWP, pore size 0.45 μm) and the filter was rinsed with 5 ml stop solution. Blanks were determined by using vesicle buffer instead of membranes in the appropriate transport media and subtracted from the results obtained by liquid scintillation counting of the filters dissolved in Optifluor® (Packard Instruments).

Experiments were performed in duplicate in a paired fashion and analyzed statistically using the paired student t-test. Differences with P values < 0.05 were considered as significant.

Results

Biochemical Characterization of the Plasma Membrane Fraction

In Table 1 the enrichment of marker enzymes in the isolated membrane fraction is compiled. The membrane fraction is highly enriched in Na-K-ATPase, and it shows high activities in alkaline phosphatase and 5' nucleotidase, markers for apical membranes in a broad variety of epithelial cells. The specific activity of marker enzymes for endoplasmic reticulum (NADH-dehydrogenase), or lysosomes (acid phosphatase) is only slightly increased in the membrane fraction. In view of the luminal localization of the Na-K-ATPase in the RPE and of the results of other investigators on fractionated RPE plasma membranes,²⁵⁻²⁶ these findings suggest that the membrane fraction is enriched in plasma membranes derived from the apical pole of the RPE. (For further considerations regarding the purity of the membranes see Discussion).

Morphological Characterization of the Plasma Membrane Fraction

In Figure 1 the results of electron microscopic investigations of the isolated plasma membrane fraction are presented. The thin section shown in Figure 1A reveals the presence of a variety of membrane fragments in the fraction, some of which have a microvillous-like appearance, some of which seem to form vesicles. The presence of vesicles in this membrane preparation is further evident from the freeze-fracture picture shown in Figure 1B. The diameter of the vesicles as determined from the freeze fracture varies between 0.2 and 0.5 μm, and the distribution

Table 1. Enrichment and recovery of enzyme activities in apical vesicle preparations from retinal pigment epithelium of the shark

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity</th>
<th>Enrichment</th>
<th>Total recovery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-K-ATPase</td>
<td>17.17 ± 1.68</td>
<td>15.2</td>
<td>125%</td>
<td>5</td>
</tr>
<tr>
<td>Alkaline</td>
<td>3.71 ± 0.18</td>
<td>10.2</td>
<td>95%</td>
<td>5</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>5.51 ± 0.76</td>
<td>10.0</td>
<td>82%</td>
<td>5</td>
</tr>
<tr>
<td>5' Nucleotidase</td>
<td>4.18 ± 0.31</td>
<td>2.2</td>
<td>94%</td>
<td>5</td>
</tr>
<tr>
<td>Mg-ATPase</td>
<td>2.96 ± 0.15</td>
<td>1.5</td>
<td>72%</td>
<td>3</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>9.91 ± 0.64</td>
<td>1.1</td>
<td>98%</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean values of n experiments are given with the standard deviations. Enrichment represents the ratio between the specific activities in μmoles/hr X mg protein found in the membrane fraction and the specific activity found in the homogenate. The recovery of protein was 95%.
Fig. 1. Electron micrograph of apical membranes isolated from shark retinal pigment epithelium. (A) Thin section. (B) Freeze fracture. The bar indicates 1 μm. (Courtesy of Dr. W. Haase, Max-Planck-Institut für Biophysik, Frankfurt/Main, FRG).
of intramembrane particles is irregular—particle-free and particle-rich areas can be observed. This phenomenon might be related to phase separation of lipids and proteins.

Orientation of the Membrane Vesicles

By comparing the ATPase activities found in freshly isolated membranes and in a membrane fraction in which all vesicles have been destroyed by freeze drying, the degree of vesiculation of the membrane vesicles can be determined. This approach takes advantage of the sidedness of the Na-K-ATPase with regard to its cytoplasmic active center of ATP-hydrolysis and its extracellular ouabain binding side.

As shown in Figure 2, freeze drying of the membrane fraction increases the Na-K-ATPase activity about 2.3-fold, whereas the activity in the presence of Mg alone or in the presence of Na, K and ouabain increases only insignificantly. Thus, in the freshly isolated membrane fraction about 58% of the Na-K-ATPase present in the membranes is not accessible to ATP. If one assumes that the isolated membranes are impermeable to ATP this result also suggests that the degree of vesiculation is at least 58%. The identity of the values obtained for the activity in the presence of Mg alone and for the activity found in the presence of Na, Na, K and ouabain indicates furthermore that all Na-K-ATPase molecules that can be reached by ATP can also be reached by ouabain. Thus no inverted vesicles appear to be present in the freshly isolated membrane fraction, as such vesicles would be expected to exhibit ouabain resistance of their Na-K-ATPase activity. In other words, all membrane vesicles seem to be oriented right-side-out and influxes measured in these membranes are identical in direction with uptake across the luminal membrane in the intact cell.

Sodium Uptake by Apical Plasma Membrane Vesicles

General characteristics: In Figure 3 the uptake of sodium into isolated apical RPE plasma membrane vesicles is shown under several conditions. In the first 10 sec of incubation at 15°C (the physiological body temperature of the shark) a very rapid association of sodium with the membrane is observed, and the rate of uptake then slows down and almost ceases between 90 and 120 min of incubation. At 0°C, where transport processes across the membrane should be slowed considerably, the initial rapid uptake is only slightly affected but the subsequent uptake is much slower than at 15°C. These results suggest that after 10 sec of incubation most of the sodium associated with the vesicles probably represents sodium bound to the membranes, whereas at later time points transport clearly can be detected. The latter time points (60 sec and 105 sec) therefore were chosen for further investigations of the characteristics of sodium transport by the RPE vesicles.

In order to obtain additional support for the notion that at least part of sodium uptake represents transport into an intravesicular space, the effect of sucrose on the uptake after 120 min was investigated. Addition of 400 mM sucrose to the incubation medium decreased uptake by 30% from 1.64 ± 0.28 pmoles/mg protein in the control to 1.14 ± 0.15 pmoles/mg protein (n = 5) in the sucrose-containing medium. Thus, sodium uptake into the vesicles occurred at least partly into an osmotically reactive space.
Also in Figure 3, the effect of amiloride on sodium uptake by the membranes was investigated. At 15°C sodium uptake into apical RPE plasma membrane vesicles is strongly inhibited by $5 \times 10^{-4} \text{ M}$ amiloride, whereas at 0°C no inhibition is observed. Thus a strongly temperature-sensitive amiloride-inhibitable sodium transport pathway can be detected in the apical RPE vesicles.

**Effect of pH gradients and amiloride concentration on sodium uptake:** Since amiloride at $5 \times 10^{-4} \text{ M}$ is known to inhibit sodium channels as well as Na/H exchanger, the action of pH gradient on sodium uptake and on the inhibition of the latter by amiloride was investigated. As shown in Figure 4, a pH gradient ($\text{pH}_1 = 6.1, \text{pH}_2 = 8.1$) across the vesicle membrane stimulates sodium uptake by 37%; this stimulation is completely blocked by $5 \times 10^{-4} \text{ M}$ amiloride. Sodium uptake in the absence of pH gradient is not inhibited by amiloride. The sensitivity to amiloride of the pH gradient-dependent sodium transport was investigated in further experiments. At $10^{-5} \text{ M}$ amiloride, where epithelial sodium channels usually are blocked completely, the inhibition of the pH gradient-dependent sodium uptake was 56±10% ($n = 4$). These data strongly suggest that the plasma membranes contain an amiloride-sensitive sodium-proton exchanger.

**Effect of valinomycin on amiloride inhibitable, pH gradient-dependent sodium uptake:** As shown in Figure 5, the amiloride-sensitive stimulation of sodium uptake by a pH gradient also can be demonstrated, when the membranes have been short-circuited by potassium and valinomycin. The stimulation of the sodium flux by the pH gradient thus cannot be attributed to an electrical potential difference developed across the membrane by the proton gradient. The small reduction of the magnitude of the Na/H antiporter activity probably is partly related to a more rapid dissipation of the proton gradient due to valinomycin-mediated K movement.

In Figure 6 the effect of sodium and lithium on the stimulation of sodium uptake by a pH gradient has been investigated. Figure 6 shows that the pH gradient-dependent uptake of $^{22}\text{Na}$ is decreased markedly in the presence of a 20-fold higher concentration of nonlabelled sodium. This tracer replacement indicates a limited number of transport sites and saturaability of the transport system. Sodium transport is inhibited to a similar extent by lithium, suggesting that lithium can replace sodium at its binding site.

**Discussion**

**Preparation of Apical Membrane Vesicles from Retinal Pigment Epithelium**

The method employed in this study to isolate apical plasma membranes from retinal pigment epithelium represents basically a modification of a technique already applied successfully to the isolation of luminal membranes from a variety of epithelial cells. It takes advantage of the higher surface charge density of the apical membranes compared to basal-lateral plasma membranes and intracellular organelles. A high charge density on the apical surface of retinal pigment epithelial cells isolated from bovine eyes has also been observed by Braunagel et al. These authors demonstrated that polylysine-coated glass beads bind retinal pigment epithelial cells by preferentially interacting with their apical pole. The purity of the membrane fraction was assessed by determination of the Na-K-ATPase, which has been shown to be predominantly present in the apical
membrane of the retinal pigment epithelium. The enrichment of Na-K-ATPase activity achieved in the final membrane preparation is identical to the one observed by Ottinello and Maraini, who isolated plasma membranes from bovine RPE by differential centrifugation followed by Percoll density gradients. This purification factor is, however, higher than that reported by Braunagel et al. The high activity of the alkaline phosphatase and 5'-nucleotidase, characteristic of luminal membranes in other transporting epithelia also identifies the isolated membranes as apical membranes. Similar to results found in isolated bovine RPE membranes, in shark RPE membranes the enrichment of alkaline phosphatase and 5'-nucleotidase is, however, lower than for Na-K-ATPase. This finding suggests that the two former enzymes are present not only in the apical RPE membrane but also in other parts of the plasma membrane or in intracellular organelles. The marker enzyme analysis and electron microscopic investigation also indicates that the plasma membrane fraction is not significantly contaminated by other cellular organelles. Since, however, in the current study marker enzymes for basal-lateral surfaces or for membranes of the Golgi complex have not been measured, a contamination of the membrane fraction with these membranes remains to be elucidated.

The major progress made in the current study is that apical membranes have been isolated as vesicles with a well defined orientation. The vesiculation is evident from the freeze-fracture pictures and from the activation of Na-K-ATPase by freeze drying. The degree of vesiculation of 60% is lower than that observed in apical membrane preparations of other epithelial cells but comparable to the vesiculation observed in contraluminal membrane fractions. Luminal membranes of rat tubule, for example, are almost completely vesiculated. The reason for this discrepancy may lie in the different organization of the apical membrane. In the dark, microvilli of the RPE retract from the rods of the retina and in scanning electron microscopy the surface of the isolated RPE is smooth with only a few microvilli left. Therefore, a higher proportion of membrane sheets rather than membrane vesicles can be expected. The orientation of the RPE apical membrane vesicles is identical to the one generally observed in isolated apical membrane vesicles.

It should be emphasized at this point that tight vesicles, into which uptake of solutes could be determined, were obtained only when calcium was present both during membrane preparation and the transport experiment. Reduction of Ca++ concentration as well as replacement by Mg++ did produce membranes that did not show an osmotically sensitive intravesicular space. Similar observations have been made for the flounder kidney brush border membrane. The reason for the calcium requirement is unknown; it should be noted, however, that the RPE normally contains high concentrations of calcium. The viability of the vesicular system in the presence of calcium made it possible to demonstrate the Na/H exchange activity.

Furthermore, it has to be considered whether the plasma membranes isolated in this study are indeed derived from the RPE or constitute components of the retinal rods which may remain attached to the RPE after the mechanical separation. In order to investigate this question, electron microscopic studies on the separated retina and the RPE were performed (data not shown). In scanning electron microscopy the basal surface of the retina shows a regular arrangement of rods, to some of which membranous material—probably residual microvilli of the RPE—is attached. The apical membrane of the RPE appears smooth, and in thin sections, very rarely rods derived from the retina can be detected. Therefore the contamination of the starting material with rods seems to be low. In addition, rods were isolated from the retina.
by mechanical agitation. These particles showed a Na-K-ATPase activity of \(1.74 \pm 0.21\) μmoles/hr \(\times\) mg protein and an alkaline phosphatase activity of \(0.184 \pm 0.06\) μmoles/hr \(\times\) mg protein; thus rods and the isolated membranes differ markedly in their enzyme content and in their enzymatic profile. However, despite the evidence accumulated above, a contribution of retinal outer plasma membranes to the data presented above cannot be completely ruled out.

Finally, it should be mentioned that not only RPE but the RPE–choroid complex was used as starting material for membrane isolation. Thus the isolated membrane fraction could also contain membranes derived from the choroid. We consider this possibility unlikely, however, since the isolation technique employed here enriches in particular luminal membranes with a high surface charge density such as microvillous membranes. The choroid located beneath the RPE is devoid of the latter structures.

Evidence for the Presence of a Na/H Exchanger in the Membrane Fraction

Several points of evidence for the presence of a Na/H exchanger in RPE apical plasma membrane were provided in this study. First, sodium uptake was stimulated by the presence of a pH gradient across the membrane. This stimulation could not be explained by electrical driving forces since it also was found in the presence of potassium and valinomycin, where transmembranal electrical potential differences should be small. The pH gradient-dependent sodium uptake was saturable and was inhibited by amiloride with an apparent \(K_i\) of \(10^{-5}\) M. In addition, lithium inhibited the pH gradient-dependent sodium uptake. All these properties resemble those described for Na/H exchange in other tissues.\(^{30,31,39}\) In isolated renal and intestinal brush border membranes a similar pH gradient leads, however, to an overshooting uptake of sodium. Such an overshoot, which is a very strong indicator for the energetic coupling of sodium and proton fluxes, could not be observed in our studies. This failure might be due to a high proton conductivity of the membrane in conjunction with a slow uptake of sodium via the Na/H exchanger. Under these conditions an overshoot would not be expected since the proton gradient would be dissipated before the sodium concentration inside the vesicles became identical to the extravesicular sodium concentration. Similar phenomena have been described for other sodium cotransport systems.\(^{40}\) It is interesting to note that in preliminary experiments when we attempted to demonstrate the Na/H exchanger by intravesicular pH measurements, a high proton permeability was observed that precluded the demonstration of sodium gradient-driven proton fluxes. Whether this high proton conductivity is due to the use of Ca instead of Mg during the membrane preparation\(^{41}\) remains to be elucidated.

Functional Role of the Na/H Exchanger in RPE

Our studies on isolated apical plasma membranes of the shark and the studies of Keller et al on cultured bovine retinal epithelial cells\(^{13,14}\) strongly suggest that RPE possesses a Na/H exchange system. Its cellular localization is probably apical; however, a presence also in the basal-lateral membranes cannot be excluded from the data currently available. The studies in bovine cells have shown that the Na/H exchanger is involved in the recovery of the cell from intracellular acidification and therefore probably plays a similar role in intracellular pH regulation, as in other epithelial and nonepithelial cells.\(^{30}\) Since the apical membrane also contains the Na-K-ATPase, recycling of sodium via the Na/H exchanger might prevent an increase in sodium concentration in the subretinal space. The effect of Na/H exchange on the pH in the subretinal space is difficult to predict in view of the lack of data on pH changes during illumination and on the actual activity of the Na/H exchanger at normal intracellular pH.

With regard to transepithelial sodium transport, the bicarbonate dependence and pH dependence of currents is compatible with the presence of a Na/H exchanger. The activity of the transport system decreases when the extracellular pH is lowered—either by directly adding protons to the incubation medium or by removing bicarbonate, which acted as extracellular buffer ion. The mechanism by which the decrease of an electroneutral transport process indirectly changes rheogenic transport in the epithelium requires further investigations.

Key words: RPE, villi, membrane vesicles, Na/H exchange, amiloride, Na-K-ATPase

Acknowledgments

The excellent technical assistance of Mariana Rubinstein is gratefully acknowledged. Mr. Charles Reign helped with the enucleations and tissue collection, and his assistance is gratefully acknowledged. The electron microscopic investigations of the intact tissue were conducted by Dr. J. Hentschel at the Center for Functional Anatomy of the Max-Planck-Institut für Systemphysiologie. We also thank Dr. W. Haase for the electron microscopical analysis of the membrane fractions.

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