An Electrophysiologic Study of Rabbit Ciliary Epithelium

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Microelectrode recordings from cells in rabbit ciliary epithelium have been made in vitro. Ionophoresis of Lucifer Yellow dye from microelectrodes during measurements of potential confirmed that the recordings were intracellular. Dye passed from the impaled cells into adjacent cells in both the nonpigmented and pigmented layers of the epithelium. Electrical coupling between epithelial cells also was observed. The mean (±SD) values of the potential measured across the basolateral membranes of the nonpigmented cells was $-65 ± 15$ mV ($n = 77$); the mean value of the input resistance at this intracellular recording site was $37 ± 28$ MΩ ($n = 17$). The membrane potential was reduced by raising the concentration of extracellular potassium but unaffected by changes in the concentrations of sodium, chloride, or bicarbonate ions. After a period of deprivation of extracellular potassium, the cells hyperpolarized without a measurable change in membrane resistance when potassium was restored to the bathing solution; this transient response to potassium was abolished by preincubation with ouabain or by bathing the epithelium in a solution lacking sodium. It was concluded that the ciliary epithelial cells are permeable to potassium but exhibit only a low permeability to sodium, chloride, or bicarbonate ions; that the cells possess an electrogenic Na/K pump; and finally, that all of the cells in the epithelium function as a syncytium. Invest Ophthalmol Vis Sci 26:371–381, 1985

The mammalian ciliary epithelium consists of a nonpigmented cell layer facing the aqueous humor and an underlying pigmented layer facing the stroma of the ciliary body. The cells of each layer are oriented such that the apical surfaces of the nonpigmented cells adjoin the apical surfaces of the pigmented layer. This bilayer forms the aqueous humor1 by ultrafiltration and active secretion,2,3 although a detailed description is not yet available. Several studies of the transport properties of the rabbit's iris-ciliary body have been made in vitro.3–8 By contrast, studies in vivo are not preferred because they are technically difficult and subject to inflammatory reactions induced by prostaglandin release in the eye.

Three reports of microelectrode recordings from rabbit ciliary epithelium have been made,9–11 but no evidence was provided by the authors to confirm that the recordings were genuinely intracellular. We have undertaken an initial electrophysiologic study of the rabbit ciliary epithelium to provide data on the value of the intracellular resting potential of both cell layers, the existence of electrical coupling between cells and the influence of ionic changes on the resting potential. This study constitutes a first attempt in a new approach to study the cellular electrophysiologic properties of this tissue.

A preliminary report has been presented to the Physiological Society.

Materials and Methods

Animals in this study were used in accordance with the ARVO Resolution on the Use of Animals in Research. Adult albino New Zealand rabbits were killed with a blow to the back of the neck. The anterior segments of both eyes were removed quickly and placed in a dish containing a modified Krebs bicarbonate Ringer solution. An iris-ciliary body was dissected from the anterior uvea as previously described.3,13–14 The isolated iris-ciliary body was pinned on to a layer of Sylgard at the bottom of a Perspex dish by four pins inserted into the choroid attached to the periphery of the preparation. A single ciliary process was dissected from the iris and transferred to a 4-ml chamber in which two monofilament...
sutures (9-0, Ethicon) were placed in a cross (Fig. 1).
The isolated process was held under the crossed sutures in order to prevent movement caused by the continuous flow of the solution (27°C) at 1 ml/min through the chamber. This flow rate was selected in order to minimize tissue disturbance during perfusion, thereby allowing increased impalement times.

### Intracellular Recording

The chamber was placed on the stage of an inverted microscope (Biovert; Reichert, Austria) and interference contrast optics at either ×40, for initial orientation, or at ×160 for microelectrode impalement. The potential was recorded between a microelectrode (40–70 MΩ), filled with 2 M potassium or sodium acetate, and a solid Ag/AgCl electrode in contact with the solution. The possibility that potassium leakage from an intracellular electrode alters the cell's potassium concentration and potential seems slight because steady potentials were recorded for long periods (30–120 min). Presumably a potassium concentration gradient would be dissipated by diffusion through the intercellular junctions (see Results). In experiments where the chloride concentration of the bathing solution was changed, the bath was earthed via a 3 M KCl saline-agar bridge. The microelectrode was connected to the input of a high impedance pre-amplifier (Model KS 700; WP Instruments, Inc., New Haven, CT), the probe head of the amplifier was held in a sliding manipulator (Carl Zeiss; Jena, East Germany). In some experiments, current pulses from a Devices stimulator triggered by a Digitimer (D4030; Devices Ltd.) were passed via a bridge circuit between the barrel of the microelectrode and the bath electrode. The bridge was balanced during the passage of current pulse (0.25 nA, 400 msec) before microelectrode penetration. Cellular impalement was achieved by...
resting the microelectrode tip on the surface of the ciliary epithelium and increasing the negative capacity compensation applied to the microelectrode so that it went briefly into electrical oscillation. After impalement when the potential had attained a steady value, the bridge balance was checked with passage of a current pulse (0.25 nA, 400 msec); frequent rebalancing was not required. Impalement acceptance depended upon achieving a rapid deflection of the recording, which either was maintained immediately or slowly increased, presumably due to sealing of the membrane around the electrode, over at least 10 min. It was not possible to obtain intracellular recordings with the lens capsule left in situ due to the rigid nature of the capsule and suspensory ligaments either preventing penetration into cells or by breaking of electrodes during attempts at impalement. This is presumably due to the convoluted nature of the tissue with many infoldings preventing microelectrode impalement.

For dye-marking experiments, microelectrodes were filled with Lucifer Yellow CH (30 mg/ml) and hyperpolarizing current pulses (10 nA, 500 msec) were passed at 1 Hz through the electrode for 10–30 min to ionophorese dye into the impaled cells. Successful impalements were judged by (1) the membrane potential per se, at about −80 mV, and (2) maintenance of cellular potential during dye ionophoresis. The latter was checked periodically and discarded if the cellular potential was less than 15% of its original, pre-ionophoresis, value. Lucifer Yellow was made up in 1 M LiCl solution, which was deposited at the electrode tip and the electrode was back-filled with 1 M LiCl. Tissues were fixed in 10% Formol saline and subsequently embedded in wax. Unstained serial 5-μm sections were examined under ultraviolet light and photographed; subsequently, the same sections were stained with hematoxylin-eosin and rephotographed to delineate the nonpigmented and pigmented cells; the latter cells lacked pigment since tissues were obtained from albino rabbits.

To determine the extent, if any, of electrical coupling between epithelial cells, two microelectrodes were inserted into the preparation at a known distance apart. A rectangular current pulse was passed through one microelectrode, and the electrotonic potentials monitored by both electrodes were recorded. The effect of varying the distance between the microelectrodes on the size of the electrotonic potential recorded by the second microelectrode was examined. The procedure for carrying out the electrical coupling measurements involved the following steps: (1) insert first microelectrode for current injection; (2) record intracellular potential of first electrode; (3) insert second microelectrode for monitoring electrotonic potentials; (4) record intracellular potential of second electrode; (5) pass rectangular current pulse through first electrode and record electrotonic potentials with both electrodes; (6) measure distance between electrode insertion sites with filar micrometer eyepiece; and (7) withdraw second electrode and repeat steps 3–7 at new site.

Permanent experimental records were obtained as pen recorder traces on a Devices M2 Recorder or as photographs of the screen of a storage oscilloscope (RM5113; Tektronix Ltd., Portland, OR).

Solutions

The modified Krebs Ringer bicarbonate solution contained (mM): NaCl, 123; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 25; glucose 25 mM. Variations of composition were: K-free solution in which KCl was omitted; low Na solution in which choline chloride replaced NaCl on an equimolar basis; Na-free solution in which choline chloride replaced NaCl and choline bicarbonate replaced NaHCO3; Cl-free solution in which sulphate replaced chloride, the calcium concentration was 2 mM, and the osmolarity was adjusted with sucrose; KCl-free solution, a modified form of Cl-free solution in which Na2SO4 replaced K2SO4; HCO3-free solution in which Na propionate replaced NaHCO3. Solutions also were used in which the [K][Cl] product was constant; these solutions had appropriate adjustment of NaCl and sucrose to keep the osmolarity constant. The pH of all solutions lay in the range 7.4–7.8, and the osmolarity was about 305 mosm/l.

In some experiments, ouabain (Strophanthin-G; Sigma Chemical Co.) was added to solutions to give a final concentration of 1, 0.1, or 0.01 mM.

Results

Intracellular Recording

In this study, microelectrodes were inserted in the ciliary epithelium across the basal surface of the nonpigmented cell layer. The insertion of a microelectrode was achieved by a brief period of electrical oscillation (see Methods) rather than by mechanical displacement. Lucifer Yellow ionophoresed from microelectrode tips during stable potential recordings was found within epithelial cells upon subsequent histologic examination (see below). During intracellular recording, the potential normally attained a steady value several minutes after impalement. Only values of the potential that remained constant (Δ < 1 mV change) for at least a further 10 min were
Fig. 2. Light and ultraviolet pictures of sections of an isolated ciliary process, which has been labelled by ionophoresis of Lucifer Yellow from an intracellular microelectrode. Fluorescent staining was found in eight adjacent sections. A, Light and corresponding ultraviolet pictures of second, fourth, fifth, and seventh sections from the series. After photography, the sections were stained with hematoxylin-eosin and rephotographed on the light microscope. B, Light and ultraviolet pictures of dye-labelled region of the eighth section showing that the Lucifer Yellow occurs in both nonpigmented and pigmented cells; this section has been stained with hematoxylin-eosin to show both cell types, nonpigmented and pigmented. The calibration bar indicates 90 μm for A and 30 μm for B. Evidently there has been some tissue shrinkage during the tissue processing. The membrane potential of the impaled cell was −88 mV and the cell was labelled by passing 10 nA pulses (500 msec) at 1 Hz for 11 min.

accepted. The input resistance did not change during the course of measurements. The mean (±SD) potential difference was −65 ± 15 mV for 77 impalements in 74 isolated processes from 26 rabbits; the distribution of values was normal. This value for intracellular potential represents impalements of both nonpigmented and pigmented epithelial cells using either potassium or sodium acetate-filled microelectrodes: no changes occurred in the cellular potential despite recording of membrane potential for up to 2 hr, thus
flux of microelectrode contents into the cells did not appear to introduce any artifacts. It was difficult to be sure of exactly which cell layer was impaled securely, although on several occasions a transient fall in potential was noted as advancement of the electrode tip was made before re-entering another zone of equal potential. In addition, as results below indicate, dye ionophoresed into one cell passes between layers; thus, it is difficult to be sure exactly which layer has been penetrated. All values in this sample were taken from microelectrode recordings that remained stable for more than 10 min. Often it was possible to record the membrane potential for periods exceeding 30 min. All potential records in this paper are labelled with the time of the recording, zero-time being taken at the moment of impalement.

Cell Coupling

Dye ionophoresed from a microelectrode during a period of stable potential recording stained not only the impaled cell but also a group of its neighbors (Fig. 2A). Presumably, the dye passed from the impaled cell through permeable junctions to adjacent cells as found in other epithelial tissues showing electrical coupling.16-18 Apparently dye also passed between nonpigmented and pigmented cells in adjacent layers and seemed to be retained preferentially in the nuclei (Fig. 2B).

Evidence of electrical coupling between cells also was obtained in four experiments. When a rectangular current pulse was passed through an intracellular electrode, an electrotonic potential could be recorded by a second intracellular microelectrode placed up to 300 μm away. The results of a representative experiment are shown in Figure 3. Each point represents a single measurement of the amplitude of the electrotonic potential, and the points are joined by straight lines for illustrative purposes. Another feature of the intracellular recordings from ciliary epithelial cells, which indirectly supports the idea that they are coupled electrically, emerged from measurements of the current/voltage relations of these cells.

Current/Voltage Relations

Rectangular current pulses passed through an intracellular electrode produced graded electrotonic potentials. The relations between current and potential change were linear for small displacements of the membrane potential (Fig. 4). The mean (± SD) input resistance obtained from such linear plots was 37 ± 28 MΩ (17 cells). A few values were well below 1 MΩ and have not been included in the sample of measurements. Due to cell coupling, the value for input resistance is an underestimate, since injected current is dissipated laterally as well as across the cellular membrane. Indeed, the time course of the charging transient and the low input resistance are indicative of more cells being charged than just the impaled cell. The epithelial cells in the ciliary processes are small (ca. 20 X 10 X 10 μm) and with a given density of ion channels per unit area, a cell will have a greater input resistance as its size decreases. Assuming a specific membrane resistance19 of 4000 Ω cm² and a cell area of 10⁻⁵ cm², the input resistance of an isolated ciliary epithelial cell would be 400 MΩ.

![Fig. 3. Typical plot of ΔV against distance (μm) for electrotonic potentials measured in one electrode relative to another electrode. The experimental arrangement is shown in the upper right inset. A current passed through the first intracellular electrode (for current injection) did not produce a conspicuous electrotonic potential at the second microelectrode before it was inserted into a cell.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933351/)
Fig. 5. Effect of extracellular potassium on the membrane potential of a ciliary epithelial cell. A, Responses of membrane potential to elevations of the potassium concentration in the bathing solution. In this and all subsequent figures, the time labelling on traces indicates the time after cellular impalement. The time course of the changes in potential reflect the mixing time in the experimental chamber at the chosen rate of superfusion of the tissue. B, A graph of membrane potential against logarithm of \([K\]_o\) for the results shown in A. The horizontal bars indicate the period of solution change.

Such high input resistances were not observed. The low values found are similar to those recorded in epithelia with extensive electrical coupling.\(^{20}\) For a nonpigmented cell with a surface area of \(10^{-5}\) \(\text{cm}^2\) and resistance of 40 \(\text{M}\Omega\), the time constant would be about 0.4 msec. However, the time constant of the electrotonic potential was about 5 msec. Thus, the relatively low input resistance and long time constant suggest that the small nonpigmented cells are coupled electrically.

### Ionic Substitutions

To establish which of the main extracellular ions influences the membrane potential of ciliary epithelial cells, a number of ionic substitutions were made in the bathing solution.

**Potassium:** Bathing an isolated ciliary process in K-free solution always led to a sustained hyperpolarization of about 5 mV below the potential noted in Krebs bicarbonate Ringer solution. The mean (± SD) value for the sustained hyperpolarization in K-free solution was \(6 \pm 5\) mV (20 cells). An increase in the potassium concentration, \([K\]_o\), caused depolarization that was graded with the size of the change in \([K\]_o\) (Fig. 5). It should be noted that the potential responses to \([K^+]_o\) were slow due to the relatively slow perfusion rate (see *Methods*). The strong dependence of potential on \([K\]_o\) suggests that the cell membrane is permeable to potassium ions. This was confirmed in a separate series of experiments where \([K\]_o\) was increased while the product \([K\]_o[C\]l\] remained constant in order to determine whether or not the response to \(K^+\) was \(\text{Cl}^-\)-dependent. A graded depolarization with increased

### Table 1. Effect of extracellular potassium concentration on the membrane potential of ciliary epithelial cells

<table>
<thead>
<tr>
<th>([K]_o) (mM)</th>
<th>Depolarization produced (mV)</th>
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<tr>
<td>9</td>
<td>8 at constant</td>
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<td>10</td>
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<tr>
<td>125</td>
<td>(49 \pm 9^*)</td>
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<td>125</td>
<td>55 at constant</td>
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<tr>
<td>125</td>
<td>52 [([K]_o[C]l]</td>
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<tr>
<td>125</td>
<td>48</td>
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Each value derived from a separate cell.

* Mean ± SD (nine cells).
[K_o] was observed again (Table 1) in general accord with the results illustrated in Figure 5 obtained at a fixed chloride concentration.

Other ions: Changes in the concentrations of sodium, chloride or bicarbonate ions in the bathing solution failed to change the membrane potentials of ciliary epithelial cells. The compositions of the solutions used in these experiments are described in Methods.

Potassium Readmission Response

It was found that, after a period of superfusion with K-free solution for at least 10 min, the readmission of Krebs bicarbonate Ringer solution always caused a transient hyperpolarization of about 10 mV lasting about 10 min. The slow time course of the response probably reflects the kinetics of mixing in the chamber. The mean (±SD) value of the hyperpolarization caused by readmission of the normal potassium concentration (4.7 mM) to K-free solution was 12 ± 6 mV (18 cells). In many cells, it was possible to repeat the potassium deprivation–readmission cycle several times and obtain consistent hyperpolarizing responses. An example shows two potassium readmission responses from a series of four responses, which had amplitudes of 16, 15, 17, and 20 mV (Fig. 6). Also shown in Figure 6 is an example of the lack of change in input resistance following readmission of potassium, although the small change expected from a change from K_o = 0 to K_o = 4.7 might not be seen easily due to current dissipation in the syncytium.

Readmission responses also were evoked by potassium concentrations above that in Krebs bicarbonate Ringer solution (four experiments) as observed previously in liver cells.21 It was notable that readmission of a solution containing [K_o] = 100 mM after superfusion with K-free solution caused a marked hyperpolarization, which lasted for about 2 min and was overtaken by a large characteristic depolarization (Fig. 7).

The potassium readmission response did not occur in ciliary processes that had been bathed in Na-free solution before and during potassium deprivation.

Effect of Ouabain

Ciliary processes were bathed in Krebs bicarbonate Ringer solution containing various concentrations of ouabain to examine whether the membrane potential has an electrogenic component. Ouabain at 0.01 mM did not change the potential of cells. When ouabain was applied at 0.1 or 1 mM during fast superfusion, it did not cause a rapid change of potential but led to a slow depolarization, which could not be reversed by superfusion with Krebs bicarbonate Ringer solution or Ringer solution with raised potassium concentration (10 mM). Even a brief exposure (2 min) to 0.1 mM ouabain was sufficient to induce a prolonged and irreversible depolarization (Fig. 8).
Fig. 7. Two examples of the readmission responses to potassium. A, Intracellular recording from a ciliary epithelial cell showing responses to potassium at 4.7 mM and 100 mM in the bathing solution. The high concentration of potassium was obtained by replacing part of sodium composition of the solution. B, Similar readmission responses recorded from a cell in another ciliary process. The time labelling on the trace indicates the time after cellular impalement.

Despite the failure of ouabain to reveal an electrogenic component of the membrane potential of cells, it was of interest to observe its effects on the potassium readmission response. Ciliary processes were superfused with K-free solution for periods of at least 20 min and then exposed for 2 min to a K-free solution containing 0.01 mM ouabain after a normal potassium readmission response had been recorded (Fig. 9). Evidently ouabain blocked the hyperpolarizing responses to potassium (readmitted at 4.7 mM or 100 mM) and the observed depolarizations were consistent with the cell's passive permeability to potassium ions.

Discussion

The results of this initial study of rabbit ciliary epithelium are at variance with those of Miller and Constant, Berggren, and Cole who reported that the nonpigmented, and pigmented cells had membrane potentials of −30 mV and −60 mV, respectively. No evidence for a group of low-potential cells has been found in our work. The difference undoubtedly reflects the improvements in microelectrode recording techniques that have occurred since the 1960s. Previous recordings were made with low resistance microelectrodes (10–20 MΩ), and probably they caused cellular damage and depolarization. We found it impossible to obtain stable recordings from ciliary epithelial cells with such low resistance electrodes. Thus, the group of low potential values previously obtained and ascribed to the nonpigmented cells by the earlier authors were probably produced by im-
palement damage. It appears that the membrane potential of both layers of ciliary epithelial cells is about \(-65\) mV and conceivably this is an underestimate of the real value because of impalement artifact expected in recordings from small epithelial cells.18 Dye-marking at the site of recording in this investigation has confirmed that the microelectrode tips were within ciliary epithelial cells. Difficulty was found, however, in discerning the precise location of the electrode tip during the majority of impalements. Thus, the mean membrane potential represents values from both cell layers. The cell-to-cell coupling, as evidenced by both dye transfer between cell layers (Fig. 2) and the electrotonic potentials, precludes against absolute definition of microelectrode tip location.

The evidence from experiments with double intracellular electrodes and dye ionophoresis indicates that the nonpigmented and pigmented cells are coupled in a functional syncytium. This finding agrees with electron microscopical studies that have revealed the presence of numerous gap junctions between ciliary epithelial cells.22 Moreover, intracellular recording demonstrates that electrical coupling is effective over distances up to 300 \(\mu\)m (Fig. 3) and dye released into an impaled cell spreads through a very large group of neighbors (Fig. 2). Probably all of the ciliary epithelial cells are equipotential and function together in generating the transport of ions from the blood to the aqueous humor. In view of the small potential difference across the ciliary epithelium,2,6,8,23 it is likely that the potential recorded across the basolateral membrane of the nonpigmented cell is almost balanced by a similar potential across the basolateral membrane of the pigmented cell.

Because the solution changes were made on the outer cell layer of the preparation and since the apical solution is probably unchanged by the experimental maneuvers, the effects of changes in the concentrations of the extracellular ions suggest that the basolateral membrane of the nonpigmented cells is permeable to potassium but less so to sodium, chloride or bicarbonate ions. Presumably the membrane potential should be close to the equilibrium potential for potassium (\(E_K\)) and more negative than the present mean estimate of the membrane potential, although lack of knowledge of the intracellular potassium concentration precludes a definitive statement on this point. If \([K]_o\) was 120 mM, which is a reasonable estimate for cellular \([K]\), then \(E_K \approx -82\) mV for \(K_0 = 4.7\) mM. Removal of extracellular potassium caused a small hyperpolarization and not a profound depolarization as reported by Berggren.9 The readmission of potassium produced a transient hyperpolarization even when \([K]_o\) was raised to 100 mM; (Fig. 7). A similar response to the readmission of 100 mM potassium has been observed in liver cells and the transient hyperpolarization attributed to an electrogenic efflux of sodium ions.21 In such experiments, the local concentration of potassium at the surface of the epithelial cells during the hyperpolarization is not known because there were diffusion delays in the bathing solutions. Nevertheless, the local concentration was probably higher than the normal value of 4.7 mM during the hyperpolarization, and thus the membrane potential must have been more negative than \(E_K\) at \([K]_o = 4.7\) mM.

Readmission responses were absent in ciliary epithelia bathed in ouabain-containing or Na-free solutions. Both conditions would prevent or reduce the active extrusion of sodium from the cells upon restoration of extracellular potassium. Similar evidence about potassium-evoked hyperpolarizations in conditions of sodium loading have been found in neurons,24,25 smooth muscle,24,26,27 liver cells,21,28 pancreatic acinar cells,29 and frog gastric mucosa30,31 (the latter called an “anomalous” response to \(K^+\)) and taken as proof of the presence of an electrogenic Na/K pump. No doubt a similar electrogenic mechanism operates in ciliary epithelial cells to extrude sodium ions during sodium loading. Evidence has been obtained in the ciliary body that the Na,K pumps at the basolateral membranes of both cell layers are electrogenic8 using \(5 \times 10^{-3}\) M ouabain, a concentra-
genic Na/K pump; and, finally, (4) all ciliary epithelial cellular potassium, which is known to interfere with to anions and sodium; (3) the cells have an electrogenic component of the resting potential of cells is about $10^5 \mu m^2$, the equivalent current density in a ciliary epithelial cell would produce a current of 10 pA. For a cell with an input resistance of 400 MΩ (see Results), this current would generate a hyperpolarization of 5 mV—a similar order to those reported here.

In ciliary processes bathed in Ringer solution, the electrogenic sodium pump apparently does not contribute to the membrane potential since ouabain did not cause a rapid depolarization upon application. Recently, Pesin and Candia have presented evidence for Na/K pumps in the basolateral membranes of both the pigmented and nonpigmented cell on the basis of effects of ouabain and amphotericin B on the short-circuit current across the epithelium. Since the action of ouabain is rapid, the failure of ouabain to depolarize cells rapidly in our experiments strongly suggests that an electrogenic Na/K pump makes little or no contribution to the membrane potential under resting conditions (see below). However, ouabain produced a slow depolarization even after only a brief application. While 0.01 mM was ineffective in causing an alteration in resting membrane potential, this concentration was effective in eliminating the transient hyperpolarization caused by passing from a K⁺-free solution to a K⁺-containing solution (Fig. 9). Thus, while a ouabain concentration of 0.1 mM is needed to interfere with the pump under resting conditions, a concentration of $10^{-2}$ M (lower than that found to induce effects on the Na/K pumps in the whole ciliary body-iris preparation) was effective in inducing changes in pump activity under conditions known to unmask the pump. It appears, therefore, that ouabain binding is virtually irreversible and, in fact, cannot be reversed by a high concentration of extracellular potassium, which is known to interfere with ouabain binding in other cells.

This initial study of the membrane potential of ciliary epithelial cells has indicated several interesting points, namely (1) the cell membrane is permeable to potassium; (2) the membrane has a low permeability to anions and sodium; (3) the cells have an electrogenic Na/K pump; and, finally, (4) all ciliary epithelial cells are electrically coupled. The contribution of these features to the role of the ciliary epithelium in the formation of aqueous humor remains to be examined. Thomas has argued on the basis of the analysis given by Mullins and Noda that the electrogenic component of the resting potential of cells in a steady state would be negligible if the sodium permeability $P_Na$ were low relative to the potassium permeability $P_K$ as found in ciliary epithelial cells. It seems likely that $P_{Na}/P_K$ for the basal membrane of the nonpigmented cells is below 0.01 and thus an electrogenic Na/K pump might contribute only about 1 mV or less to the resting potential (see Fig. 2, Thomas). In the nonsteady state, however, when cells are sodium-loaded, the enhanced pump activity could lead to a hyperpolarization as large as those reported here and elsewhere (see above).

Key words: rabbit, ciliary epithelium, intracellular potential, Na/K pump, ionic changes, cell-cell coupling

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