J. Membrane Biol. 44, 281–307 (1978)

Effect of Intracellular Potassium upon the Electrogenic Pump of Frog Retinal Pigment Epithelium

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Received 4 April 1978; revised 8 August 1978

Summary. We have studied the hyperpolarizing, electrogenic pump located on the apical membrane of the retinal pigment epithelium (RPE) in an *in vitro* preparation of bullfrog RPE-choroid. Changes in RPE $[K^+]_i$ alter the current produced by this pump. Increasing $[K^+]_o$ in the solution perfusing the *basal* membrane increases RPE $[K^+]_i$ (measured with a K^+-specific microelectrode), and also depolarizes the *apical* membrane. This depolarization is due to a decrease in electrogenic pump current flowing across the apical membrane resistance, since it is abolished when the pump is inhibited by apical ouabain, by cooling the tissue, or by 0 mM $[K^+]_o$ outside the apical membrane. Removal of Cl^- from the solution perfusing the basal membrane abolishes the K^+-evoked apical depolarization by preventing the entry of K^+ (as KCl) into the cell. We conclude that the increase in $[K^+]_i$ causes the decrease in pump current. This result is consistent with the finding that $[K^+]_i$ is a competitive inhibitor of the $Na^+ - K^+$ pump in red blood cells.

It is possible that the light-evoked changes in $[K^+]_o$ in the distal retina could alter RPE $[K^+]_i$, and thus could affect the pump from both sides of the apical membrane. Any change in pump current is likely to influence retinal function, since this pump helps to determine the composition of the photoreceptor extracellular space.

In the retinal pigment epithelium (RPE), there is a ouabain-sensitive pump located on the apical membrane (the membrane facing the photoreceptors) that actively transports sodium ions across the RPE in the choroid-to-retina direction (Miller & Steinberg, 1977*b*). In the preceding paper, we presented electrophysiological evidence that this pump is electrogenic, and contributes approximately −10 mV to the resting

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0022-2631/78/0044-0281 $05.40

potential of the apical membrane (Miller, Steinberg & Oakley, 1978). This electrogenic pump is further characterized in the present study.

In the course of an earlier study (Miller & Steinberg, 1977*a*), it was noticed (*unpublished*) that increasing extracellular potassium ion concentration, $[K^+]_o$, outside the *basal* membrane (the membrane facing the choroid) caused a depolarization of the *apical* membrane, which was larger in magnitude than could be explained by the passive electrical analogue of the RPE. In addition, this "excessive" apical depolarization (produced by elevating basal $[K^+]_o$ for more than 3 min) was seen to be abolished by applying ouabain to the apical membrane or by cooling the tissue. These results suggested that the excessive depolarization was caused by a decrease in hyperpolarizing pump current flowing across the apical membrane resistance. It was hypothesized that the increase in basal $[K^+]_o$ caused an increase in RPE intracellular potassium ion concentration, $[K^+]_i$, which inhibited the apical electrogenic pump.

The purpose of this paper is to confirm and to extend those earlier observations and to test directly the K^+ hypothesis by measuring RPE $[K^+]_i$ with K^+-specific microelectrodes. These measurements show that an increase in $[K^+]_i$ is associated with the decrease in pump current. Thus, it seems likely that the electrogenic pump is inhibited directly by the increase in $[K^+]_i$.

Materials and Methods

Preparation and Solutions

The techniques used to dissect the pigment epithelium-choroid preparation, and to mount this tissue as a membrane separating two perfusion chambers, have been described previously (Miller & Steinberg, 1977*a*). Each perfusion chamber was named for the pigment epithelial membrane it faced: *apical* or *basal*. The tissue was perfused continuously on each side, usually with a modified Ringer's solution having the following composition (in mM): 82.5 NaCl, 2.0 KCl, 27.5 $NaHCO_3$, 1.0 $MgCl_2 \cdot 6\,H_2O$, 1.8 $CaCl_2$, and 10.0 glucose. This solution was bubbled continuously with 95% O_2/5% CO_2, and had a pH of 7.4±0.1. Membrane depolarizations were produced by a modified Ringer's solution having a $[K^+]$ of 55 mM, in which KCl replaced NaCl on an equimolar basis. In one experiment, the solutions were altered to have 0 mM Cl^-, by substituting Na methylsulfate for NaCl, K methylsulfate for KCl, $MgSO_4$ for $MgCl_2 \cdot 6\,H_2O$, and $CaSO_4$ for $CaCl_2$. In another experiment, the solutions were altered to have 0 mM HCO_3^-, by substituting Na Methylsulfate for $NaHCO_3$. In this case, a phosphate buffer was used (2.125 mM Na_2HPO_4, 0.375 mM $NaH_2PO_4 \cdot H_2O$), and the solutions were bubbled with 100% O_2.

Electrodes

Double-barrel, potassium-specific microelectrodes for intracellular recording were made using a liquid ion-exchanger solution (Corning #477317), as described previously (Oakley, 1977), with several modifications. At the electrode tip, the opening of the reference barrel was within 0.1 μm of the opening of the ion-specific barrel. Leakage of cations from the reference barrel, therefore, might be sensed by the ion-specific barrel. To prevent this, the reference barrel was filled with 5.0 M LiCl, since the ion-exchanger solution is relatively insensitive to Li^+ (Wise *et al.*, 1970). In addition, the electrodes were bevelled on a surface embedded with 0.05 μm alumina particles (Brown & Flaming, 1975), while the resistance of the reference barrel was monitored. After bevelling, the resistance of the reference barrel ranged from 100 to 200 MΩ.

Recording

The recording system, shown schematically in Fig. 1, was similar to that described by Miller and Steinberg (1977*a*) and Oakley (1977). The transepithelial potential (TEP) was recorded (apical side positive) differentially across the tissue. Membrane potentials from pigment epithelial cells were recorded by micropipette electrodes, with respect to either the apical or basal solution. When referred to the apical solution, the voltage measured was the apical membrane potential, V_{AP}, and when referred to the basal solution, the voltage measured was the basal membrane potential, V_{BA}.

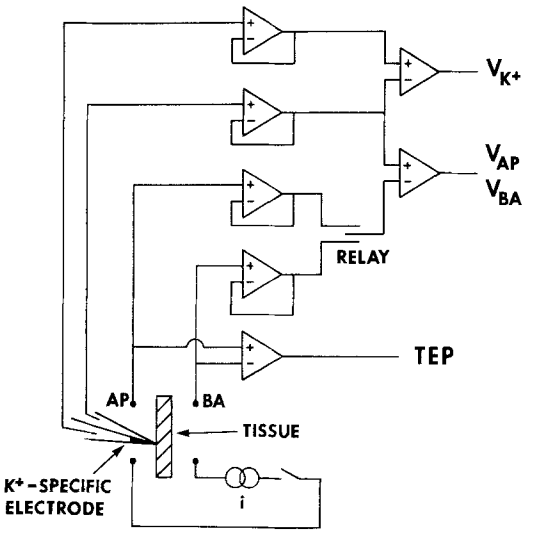

Fig. 1. A schematic diagram of the system used to record the transepithelial potential (TEP), the apical and basal membrane potentials (V_{AP} and V_{BA}, respectively), and the differential potential (V_{K+}) between the two barrels of the intracellular, K^+-specific microelectrode. The relay was switched every 100 msec, to record V_{AP} and V_{BA} on a single channel. In some figures, the combined (V_{AP}, V_{BA}) signal is displayed on a slow time base, so that V_{BA} and V_{AP} form the upper and lower envelopes, respectively, of the waveform. In other figures, the individual waveforms, V_{AP} and V_{BA}, have been extracted from FM recordings of the combined signal by using a digital computer. The equivalent resistance of the tissue could be measured by passing constant current pulses (i) across the tissue (Miller & Steinberg, 1977*a*)

In some experiments, pigment epithelial membrane potentials and $[K^+]_i$ were measured simultaneously with a double-barrel, K^+-specific microelectrode, as shown in Fig. 1. The membrane potentials were measured through the reference barrel, and a potential (V_{K+}) proportional to the logarithm of $[K^+]_i$ was measured differentially between the two barrels (ion-specific barrel positive). The preamplifier used to measure the potential from each barrel was capacity compensated, and had an input resistance of $\simeq 10^{14}\,\Omega$.

The potentials were measured on an oscilloscope (Tektronix) and digital millivoltmeters (Weston). For all of the illustrated responses, the potentials were first recorded on an FM tape recorder (Vetter C4, A.R. Vetter Co.) and then digitized and plotted by a computer (Nova 2, Data General Corp.; equipped with a Zeta plotter, Model 100, Zeta Research, Inc.).

Measurement of $[K^+]_i$

The differential potential (V_{K+}) between the two barrels of a double-barrel, K^+-specific microelectrode can be described empirically as

$$V_{K^+} = \frac{2.303\,nRT}{F} \log_{10}\left([K^+] + \frac{[Na^+]}{S}\right) + V_0 \tag{1}$$

where n is a constant (<1.0) accounting for the nonideal slope of the electrode, R is the gas constant, T is the absolute temperature, F is the Faraday number, S is the selectivity coefficient for K^+ over Na^+, and V_0 is a constant potential (Walker, 1971; Oakley & Green, 1976; Oakley, 1977). The term $\frac{2.303\,nRT}{F}$ is referred to as the electrode slope, abbreviated α (Kimura & Fujimoto, 1977). The electrodes were calibrated in modified Ringer's solutions, in which the sum of $[K^+]$ and $[Na^+]$ was held constant at 112 mM. Since these solutions were of constant ionic strength, the potassium ion activity coefficient was constant in each solution (Robinson & Stokes, 1968). The three parameters of Eq. (1) (α, S, V_0) were fit to the electrode calibration data using a log-linear, least-squares regression analysis. A typical, computer-generated, calibration curve is shown in Fig. 2.

Equation (1) is valid whether the electrode is placed extracellularly or intracellularly. Upon rearranging Eq. (1), intracellular $[K^+]$ is given by

$$[K^+]_i = 10^{(V_{K^+} - V_0)/\alpha} - \frac{[Na^+]_i}{S}. \tag{2}$$

This equation states that $[Na^+]_i$ must be known in order to solve for $[K^+]_i$. However, the average value of S was 36 (10 electrodes), so if $[Na^+]_i$ is <36 mM (which it likely is, based on the data of Miller and Steinberg, 1977*a*), the term $[Na^+]_i/S$ would contribute <1 mM to Eq. (2). Consequently, this term was ignored, and $[K^+]_i$ was computed as

$$[K^+]_i = 10^{(V_{K^+} - V_0)/\alpha}. \tag{3}$$

The sensitivity of the electrode potential (V_{K^+}) to changes in $[K^+]_i$ can be calculated by differentiating Eq. (3) with respect to V_{K^+}, yielding

$$\frac{d[K^+]_i}{dV_{K^+}} = \frac{2.303}{\alpha} 10^{(V_{K^+} - V_0)/\alpha} = \frac{2.303}{\alpha}[K^+]_i. \tag{4}$$

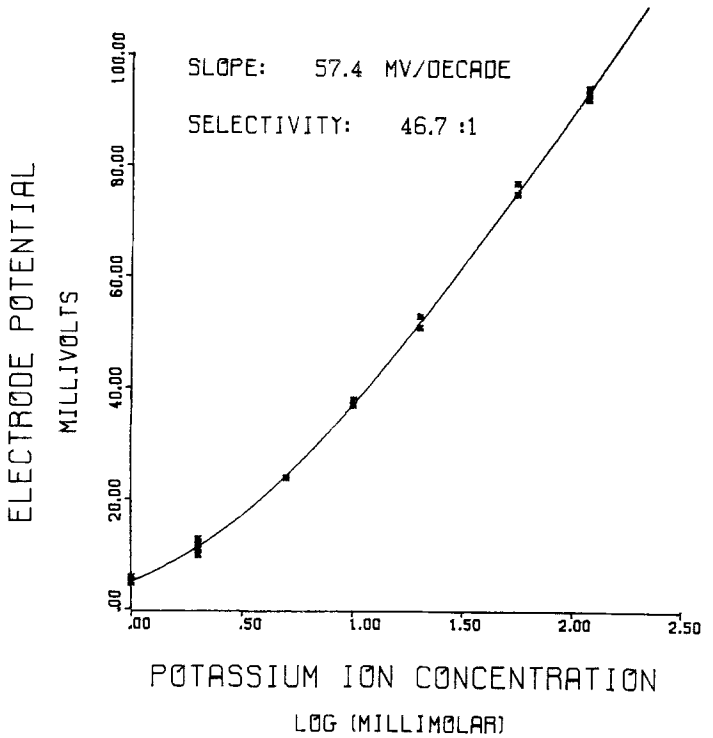

Fig. 2. A calibration curve for a K^+-specific microelectrode. The electrode potential (V_{K^+}) is plotted as a function of the potassium ion concentration (logarithmic scale) in the calibration solutions. In these solutions, the sum of $[K^+]$ and $[Na^+]$ was held contant, equal to 112 mM. The solid curve is of the form of Eq. (1), and was calculated by using a log-linear, least-squares regression analysis. The electrode slope (α) was 57.4 mV per decade change in $[K^+]$, and its selectivity (S) for K^+ over Na^+ was 46.7: 1

The average value of α was 58.3 mV/decade (10 electrodes), and the average resting value of $[K^+]_i$ was 121 mM (14 cells, *see Results*). Thus, the term $d[K^+]_i/dV_{K^+}$ was $\simeq 4.8$ mM/mV, indicating that at the intracellular concentration of potassium, the K^+-specific microelectrodes were relatively insensitive to changes in $[K^+]_i$.

Sources of Error in the Measurement of $[K^+]_i$

One of the major problems with intracellular, ion-specific microelectrodes is that the resistance of the ion-specific barrel is extremely high. The specific resistivity of the Corning potassium ion-exchanger solution is $\simeq 2{,}000$ times greater than that of 2 M KCl (Zeuthen, Hiam & Silver, 1974). Thus, a micropipette, which has a resistance of 100 MΩ when filled with 2 M KCl, will have a resistance of $\simeq 2 \times 10^{11}\ \Omega$ when made into an ion-specific microelectrode. In addition, there is a large capacitance associated with these elec-

trodes, since they are used in a perfusion chamber where more than 1 cm of the electrode tip must be submerged in electrolyte. If the electrode capacitance is 50 pF (in parallel with the electrode resistance of $2 \times 10^{11}\,\Omega$), the electrode RC time constant will be 10 sec. The reference barrel of the double-barrel, K^+-specific microelectrode has a much lower resistance (100–200 MΩ when filled with 5 M LiCl) and thus has a much smaller time constant (< 10 msec). Consequently, whenever the membrane potential changes rapidly, the ion-specific barrel will record a distorted electrical response, and the differential voltage (V_{K+}) between the two barrels (which should be a measure of $[K^+]_i$ only) will contain an electrical artifact.

In order to prevent this type of artifact, which would have been sensed as a change in $[K^+]_i$, it was necessary to use a capacity-compensated preamplifier for the ion-specific barrel. Increasing the "negative" capacitance decreased the effective electrode capacitance, and thus decreased the electrode time constant. An experiment was designed to check the effectiveness of the capacity compensation.

The tip of a double-barrel, K^+-specific microelectrode was placed into the apical perfusion bath. Constant current pulses of 10 sec duration were passed across the pigment epithelium-choroid preparation in the basal-to-apical direction. This current produced a voltage drop across the tissue, decreasing the potential of the apical chamber with respect to ground. As shown in Fig. 3*A*, the response of the uncompensated, ion-specific barrel (V_{ION}) to this voltage change was typical of an electrode with a very large RC time constant. Since the reference barrel (V_{REF}) responded much faster to the voltage change (note that $-V_{REF}$ is plotted), the differential signal between the two barrels ($V_{K+} = V_{ION} - V_{REF}$) contained a large artifact. When the ion-specific barrel was optimally compensated, as shown in Fig. 3*B*, it responded much more rapidly to the voltage change. The differential potential (V_{K+}) contained a much smaller electrical artifact. Due to distributed electrode capacitance, however, the electrode could not be perfectly compensated. Thus, V_{K+} will contain an electrical artifact whenever the potential at the tip changes in a stepwise manner. Since the membrane potential changes in the pigment epithelium develop over 20–100 sec, the electrical artifact in V_{K+} should be minimal when the electrode is properly compensated.

Another problem with intracellular, K^+-specific microelectrodes is in the measurement of absolute values of $[K^+]_i$. The value of $[K^+]_i$ is related to V_{K+} by the electrode calibration curve (Fig. 2). Drift in the potential of either the reference barrel or the ion-specific barrel, between the time the electrode is calibrated and the time that it is used, will introduce an error into the measurement. This error is approximately 4.8 mM/mV (*see* above). In addition, changes in tip potential during the intracellular measurement, due to tip bending or breakage, will introduce a similar error. These errors were minimized by calibrating the electrodes both before and after an experiment, and only accepting measurements when the electrode drift during the entire experiment was less then 2 mV.

When the electrode tip penetrates a cell membrane, ideally both barrels should sense the same membrane potential, so that the differential potential between the two barrels (V_{K+}) will not be contaminated by the membrane potential. Since the tip of the electrode is $\simeq 0.3\,\mu m$ in diameter, it is likely that both barrels of the electrode are at an intracellular position of equal potential. If the resistance of the ion-specific barrel, however, is as large as $10^{12}\,\Omega$, 1% of the cell's membrane potential could drop across the electrode itself (the preamplifier had an input resistance of $10^{14}\,\Omega$). Since pigment epithelial cells have large membrane potentials ($V_{AP} = -88$ mV, Miller & Steinberg, 1977*a*), this could lead to a difference of almost 1 mV between the membrane potentials sensed by each barrel, and thus a 1 mV error in V_{K+} and a 4.8 mM error in $[K^+]_i$.

The calculated absolute value of $[K^+]_i$ also could be affected by an interfering cation within the cell. The ion-exchanger solution used has appreciable sensitivity to Cs^+, Rb^+, and certain quaternary ammonium ions (Wright & McDougal, 1972; Neher & Lux,

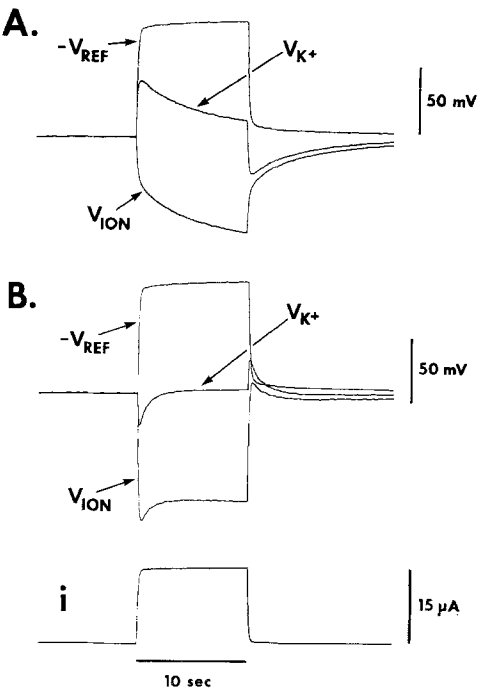

Fig. 3. The response of a double-barrel, K^+-specific microelectrode to a step change in voltage. The tip of the electrode was placed in the apical chamber, and a 14-μA current pulse (labelled *i*) was passed across the tissue in the basal-to-apical direction for 10 sec. This current pulse produced a voltage drop across the tissue resistance. Since the basal chamber was held at virtual ground, the voltage drop decreased the potential of the apical chamber with respect to ground. The potential of the ion-specific barrel (V_{ION}) and the potential of the reference barrel (V_{REF}) were both measured with respect to ground (note that $-V_{REF}$ is plotted). The differential potential between the two barrels (V_{K+}, equal to $V_{ION}-V_{REF}$) was also measured. (*A*): With no negative capacitance. (*B*): With a maximum amount of negative capcitance applied to the ion-specific barrel

1973). The absolute error produced by these interfering cations could not be measured. In addition, there could be an intracellular protein that could affect the selectivity of the ion-exchanger solution, although the electrode response is not affected by proteins in blood or cerebrospinal fluid (Wise, Kurey & Baum, 1970).

One other source of error must be considered. The tips of the double-barrel, K^+-specific microelectrodes were much larger in diameter than the tips of the single-barrel microelectrodes used previously (Miller & Steinberg, 1977*a*). In many penetrations, the electrode seemed to poke a large hole in the cell membrane. Immediately after penetration, both the membrane potential and V_{K+} decreased in magnitude, necessitating removal of the electrode from the cell. The data reported in this paper were from cells whose membrane potential and $[K^+]_i$ did not decrease in magnitude after cell penetration. In these cells, V_{AP} averaged -85 mV (14 cells, 9 tissues).

Results

Perfusion with High $[K^+]$ *Solutions*

The apical and basal membranes both have a significant relative potassium conductance; perfusion of either membrane with a high $[K^+]$ Ringer's solution, therefore, will depolarize that membrane by decreasing the magnitude of the K^+ equilibrium potential (Miller & Steinberg, 1977*a*). Since both membranes are electrically coupled via a shunt resistance, a K^+-induced depolarization of either membrane will change the current flowing across both membranes and will thus change the potential measured across each membrane (Miller & Steinberg, 1977*a*; Miller, Steinberg & Oakley, 1978). As a result of the shunt pathway, both membranes depolarize, even though only one membrane is perfused with the high $[K^+]$ solution.

Apical Membrane

The effects of perfusing the apical membrane with high $[K^+]$ Ringer's solution are shown in Fig. 4 (left side). At the start of the trace, the solution entering the apical chamber was switched from standard

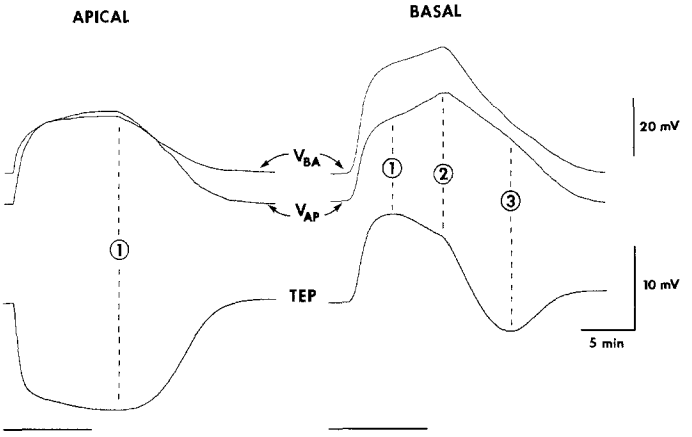

Fig. 4. Changes in V_{AP}, V_{BA}, and TEP as a result of perfusion with high $[K^+]$ Ringer's solution. The horizontal line under each set of responses indicates the time during which the high $[K^+]$ solution flowed into the chamber, either the apical chamber (left) or the basal chamber (right). These responses were recorded sequentially from the same cell. The changes in membrane potential (upper traces) were superimposed upon a V_{AP} of -88 mV, and the changes in TEP were superimposed upon a steady level of $+10$ mV. During the apical response (left), the apical membrane became slightly depolarized with respect to the basal membrane, thereby making the TEP negative

Ringer's solution to high $[K^+]$ Ringer's solution. The horizontal bar below the responses indicates the time interval during which the high $[K^+]$ solution was entering the chamber. Due to convective flow through the perfusion system and diffusion through the unstirred layer there was a delay before the high $[K^+]$ solution actually arrived at the apical membrane (Miller *et al.*, 1978). After this delay, the high $[K^+]$ solution depolarized the apical membrane. Due to the shunt pathway, the depolarization of the apical membrane caused the basal membrane to depolarize. From the beginning of the response until time *1*, the magnitude of the depolarization was greater at the apical membrane than at the basal membrane, so the TEP (equal to $V_{BA} - V_{AP}$) decreased and became slightly negative. After the solution perfusing the apical membrane was switched back to the standard Ringer's solution, the membranes repolarized, and the TEP increased monotonically towards its original value. These reponses are consistent with the passive shunt model of the RPE (*see Appendix*).

Basal Membrane

The changes in membrane potential in response to changing basal $[K^+]_o$ were more complicated than those recorded in response to changing apical $[K^+]_o$, as shown in Fig. 4 (right side). When the high $[K^+]$ Ringer's solution was applied to the basal membrane (via the choroid), the basal membrane depolarized. The latency of the membrane depolarization was longer than that observed during the apical response. This longer latency was likely due to the choroid acting as a diffusion barrier, increasing the width of the unstirred layer that separates the basal membrane from the fluid in the basal chamber (Miller & Steinberg, 1977*a*).

Due to the shunt pathway, the depolarization of the basal membrane caused a depolarization of the apical membrane. From the beginning of the response until time *1*, the rate of depolarization was greater at the basal membrane than at the apical membrane, so the TEP increased. Between times *1* and *2*, however, the rate of depolarization was greater at the apical membrane than at the basal membrane, so the TEP decreased. After the standard Ringer's solution was re-introduced, the basal membrane hyperpolarized, and the apical membrane also hyperpolarized. Between times *2* and *3*, the rate of hyperpolarization of the basal membrane was greater than that of the apical membrane, so the TEP

decreased. The value of the TEP reached a minimum at time *3*. After time *3*, the rate of hyperpolarization was greater at the apical membrane than at the basal membrane, and the TEP increased towards its original value. Unlike the response to the change in apical $[K^+]_0$, the TEP did not return monotonically to its original value following re-introduction of the standard Ringer's solution to the basal chamber. The TEP first decreased below its original value (an overshoot), and then slowly returned to its original value.[1]

Smaller increases in basal $[K^+]_o$ produced smaller but similar effects. For an increase in basal $[K^+]_o$ from 2 to 10 mM, for example, the effect was approximately one-fifth the size produced by an increase from 2 to 55 mM.

In terms of passive electrical shunting, it is not possible to account for the changes in apical membrane potential observed during perfusion of the basal membrane with high $[K^+]$ solution (a quantitative analysis of these changes in potential is given in the *Appendix*). In Fig. 4 (right side), for example, between times *1* and *2* the apical membrane was *depolarizing* at a greater rate than the basal membrane, and after time *3* the apical membrane was *hyperpolarizing* at a greater rate than the basal membrane.

The responses in Fig. 5 provide additional evidence for the notion that the changes in apical membrane potential are not due solely to passive electrical shunting. This figure illustrates the changes in membrane potential resulting from perfusion of the basal membrane with high $[K^+]$ Ringer's solution, but for a longer time ($\simeq$20 min) than in Fig. 4. At the start of the illustrated waveforms, the solution perfusing the basal membrane was switched from standard Ringer's solution to high $[K^+]$ Ringer's solution. Initially, the high $[K^+]$ solution depolarized the basal membrane, and due to the change in shunt current, the apical membrane also depolarized. From the beginning of the response until time *1*, the basal membrane depolarized at a greater rate than the apical membrane, so the TEP increased. After time *1*, however, the apical membrane

1 The membrane potential changes were produced by a modified Ringer's solution having a $[K^+]_o$ of 55 mM, in which KCl replaced NaCl on an equimolar basis. In order to demonstrate that the observed effects were not caused by a *reduction* in $[Na^+]_o$, the basal membrane was perfused for 15 min with a modified Ringer's solution, in which either 53 mM choline chloride or 53 mM Tris chloride was substituted for 53 mM NaCl. Basal $[K^+]_o$ was then increased from 2 to 55 mM by exchanging 53 mM KCl for the Tris chloride or the choline chloride. In both cases, the TEP response was identical to the control response obtained by exchanging NaCl for KCl. The observed potential changes, therefore, must have been due to the increase in basal $[K^+]_o$.

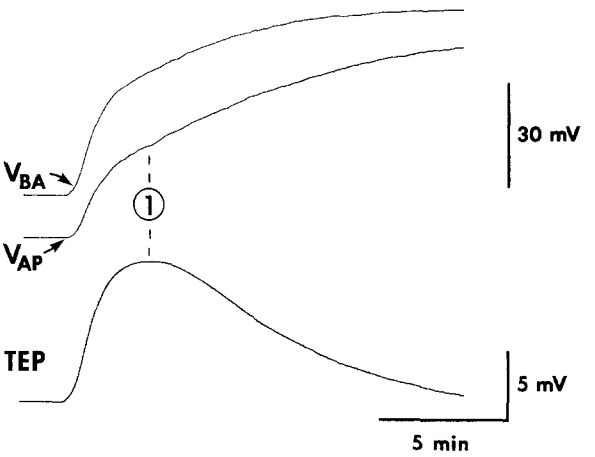

Fig. 5. Changes in V_{AP}, V_{BA}, and TEP as a result of perfusion of the basal membrane with high $[K^+]$ Ringer's solution. At the beginning of the trace, the solution perfusing the basal membrane was switched to the high $[K^+]$ solution; this solution continued to flow into the basal chamber for the duration of the time interval illustrated. These responses were superimposed upon a V_{AP} of -88 mV and a TEP of $+10$ mV, and were recorded from the same cell as in Fig. 4

depolarized at a greater rate than the basal membrane, so the TEP decreased. By the end of the time interval illustrated in the figure, the TEP had decreased almost to its original value, and at this time, the basal membrane had essentially ceased to depolarize. The apical membrane, however, was still depolarizing. Again, the changes in apical membrane potential (after time *1*) cannot be explained on the basis of passive electrical shunting of a change in potential generated at the basal membrane.

It was hypothesized that the excessive depolarization of the apical membrane was due to a decrease in the current from the hyperpolarizing, electrogenic pump. Since the major fraction of the pump current flows across the apical membrane, any decrease in the pump current would immediately depolarize the apical membrane more than the basal membrane (Miller *et al.*, 1978). Thus, in Fig. 5, a decreasing pump current would explain why, after time *1*, the apical membrane depolarized at a greater rate than the basal membrane.

Inactivation of the Electrogenic Pump

If the above hypothesis is valid, then inactivation of the pump by ouabain, cold, or 0 mM $[K^+]_o$ outside the apical membrane (Miller *et al.*,

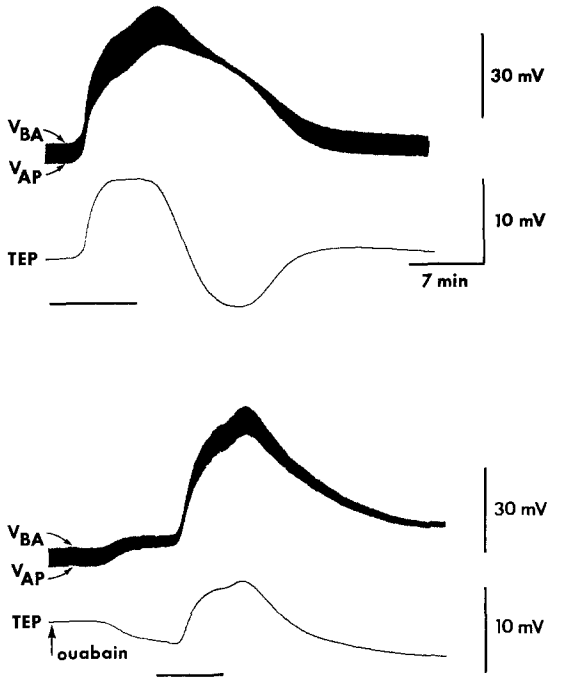

Fig. 6. Effects of apical ouabain on the K^+-evoked changes in V_{BA}, V_{AP}, and TEP. The basal membrane was perfused with high $[K^+]$ Ringer's solution for the time periods indicated by the horizontal bars. These responses were recorded sequentially from the same cell, and were superimposed upon a V_{AP} of -88 mV and a TEP of $+10.2$ mV. *Upper:* Control responses. *Lower:* Ouabain responses. Beginning at the time indicated by the arrow, the apical membrane was continuously perfused with ouabain (0.1 mM) Ringer's solution

1978) should prevent the excessive changes in apical membrane potential that result from perfusing the basal membrane with high $[K^+]$ solution. At the top of Fig. 6, control changes in membrane potential and TEP are shown that are characteristic of those seen during a change in basal $[K^+]_o$. Similar responses were recorded from the same cell, as shown in the lower half of Fig. 6, except at the start of these lower traces, the solution perfusing the apical membrane was switched to a Ringer's solution containing 10^{-4} M ouabain. During the subsequent perfusion of the basal membrane with high $[K^+]$ Ringer's solution (horizontal bar), the excessive changes in apical membrane potential were absent. The changes in apical membrane potential in the presence of ouabain can be completely explained by passive electrical shunting (*see Appendix*). The K^+-evoked changes in TEP after ouabain reflected the absence of the excessive apical depolarization. The TEP first increased, and following

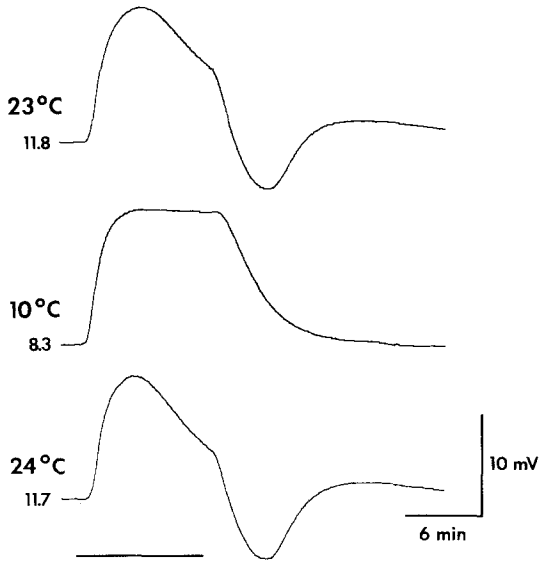

Fig. 7. Effects of cooling the tissue on the K^+-evoked changes in TEP. The basal membrane was perfused with high $[K^+]$ Ringer's solution for the time periods indicated by the horizontal bar. These responses were measured sequentially from the same preparation. The number at the left of each trace is the value of the TEP at the start of the trace. *Top:* control response, 23 °C. *Center:* response after cooling, 10 °C. *Bottom:* response after warming, 24 °C

the return to perfusion with the standard Ringer's solution, it decreased monotonically towards its original value.

It is apparent from the responses in Fig. 6 that monitoring the changes in TEP is sufficient to determine if a particular experimental protocol abolishes the excessive depolarization of the apical membrane. Thus, it was not always necessary to record the changes in membrane potential.

It was possible to inhibit the electrogenic pump by cooling the tissue (Miller, Steinberg & Oakley, 1978). This procedure also altered the changes in TEP measured during perfusion of the basal membrane with high $[K^+]$ solution. The changes in TEP were first measured with the tissue at room temperature (23 °C), as shown at the top of Fig. 7. The tissue was then cooled by perfusion of both membranes with cold Ringer's solution, so that its temperature, measured by a thermistor in the basal chamber, fell to 10 °C. Cooling the tissue decreased the TEP by 3.5 mV (the absolute value of the TEP in mV is shown at the left of each trace). The basal membrane was then perfused with high $[K^+]$ Ringer's solution (cold), and the changes in TEP were measured, as shown by the

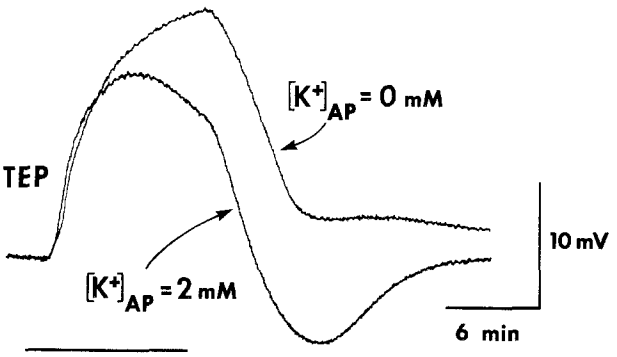

Fig. 8. Effects of 0 mM $[K^+]_o$ (apical) on the K^+-evoked changes in TEP. The control response to perfusion of the basal membrane with high $[K^+]$ Ringer's solution (horizontal bar) was recorded first, while the apical membrane was perfused with the standard Ringer's solution (labeled $[K^+]_{AP}=2$ mM). The apical membrane was then perfused with 0 mM $[K^+]$ Ringer's solution for 20 min, and the TEP fell from +10.3 to +5.6 mV. While continuing to perfuse the apical membrane with 0 mM $[K^+]$ Ringer's solution, the basal membrane was perfused with high $[K^+]$ Ringer's solution (horizontal bar), and the change in TEP was recorded (labeled $[K^+]_{AP}=0$ mM)

middle response in Fig. 7. Consistent with the electrogenic pump hypothesis, cooling the tissue by 13 °C abolished the excessive depolarization of the apical membrane, as inferred from the changes in TEP. The effects of cold were reversible, as shown by the response at the bottom of Fig. 7.

The electrogenic pump also could be inactivated by removal of K^+ from the solution perfusing the membrane on which the pump is located (Glynn, 1962; Skou, 1975; Schwartz, Lindenmayer & Allen, 1975; Miller *et al.*, 1978). The effects of 0 mM $[K^+]_o$ (apical) on the K^+-evoked changes in TEP were measured as follows. The tissue was initially perfused on both sides with the standard Ringer's solution. As shown in Fig. 8, when the basal membrane was perfused with high $[K^+]$ Ringer's solution for 10 min, the change in TEP showed the typical overshoot pattern. Following this control response, the solution perfusing the apical membrane was switched to a Ringer's solution containing 0 mM $[K^+]$ (NaCl substituted for KCl). After 25 min, the TEP had decreased from 13.0 to 6.8 mV. The basal membrane was again perfused with high $[K^+]$ Ringer's solution for 10 min, during which time the apical membrane was continuously perfused with 0.0 mM $[K^+]$ solution. It was inferred from the waveform of the change in TEP that this procedure greatly reduced the excessive depolarization of the apical membrane. The effects of 0.0 mM $[K^+]$ were reversible (not shown).

It can be concluded that conditions that decrease the pump current, or inactivate the pump altogether, also abolish the excessive changes in apical membrane potential. The data support the hypothesis that these changes in potential are caused by changes in current from the electrogenic pump, even though these changes in current are elicited by a change in $[K^+]_o$ outside the basal membrane.

It is possible that perfusion of the basal membrane with high $[K^+]$ Ringer's solution increases $[K^+]_i$, and that this increase in $[K^+]_i$ directly inhibits the pump. In red blood cells, for example, several studies indicate that K^+ competes with Na^+ for pump sites on the *inside* of the cell membrane, so that an increase in $[K^+]_i$ decreases the amount of Na^+ pumped out of the cell (Garay & Garrahan, 1973; Knight & Welt, 1974; Simons, 1974; Bodemann & Hoffman, 1976; Blostein & Chu, 1977). Thus, the K^+ ions that move across the *basal* membrane could decrease the electrogenic pump current by competitive inhibition from the inside of the *apical* membrane. It was hypothesized, therefore, that the changes in electrogenic pump current observed during changes in basal $[K^+]_o$ were caused by changes in $[K^+]_i$.

Measurement of $[K^+]_i$

Potassium-specific microelectrodes were used to measure $[K^+]_i$ directly. A K^+-specific microelectrode was advanced towards the pigment epithelium from the apical side. Penetration of the apical membrane was indicated as the membrane potential was sensed by the reference barrel. Simultaneously, the high intracellular $[K^+]$ was sensed as an increase in V_{K^+}. The value of V_{K^+} was converted into a value of $[K^+]_i$ by using Eq. (3). In 14 cells from 9 tissues, the resting level of $[K^+]_i$ was 121 ± 5 mM (mean $\pm$ SEM).

Changes in V_{AP}, V_{BA}, V_{K^+}, and TEP that were recorded during perfusion of the basal membrane with high $[K^+]$ Ringer's solution are shown in Fig. 9. The membrane potentials were recorded by the reference barrel, with respect to either the apical or basal solution (*Materials and Methods*, Fig. 1). Perfusion of the basal membrane with the high $[K^+]$ solution caused an increase in V_{K^+}, indicating an increase in $[K^+]_i$. During the entire time that the basal membrane was perfused with the high $[K^+]$ solution, $[K^+]_i$ continued to increase. It can be inferred that $[K^+]_o$ outside the basal membrane was also continuing to increase during this time, since measurements of $[K^+]_o$ in the choroid, made with

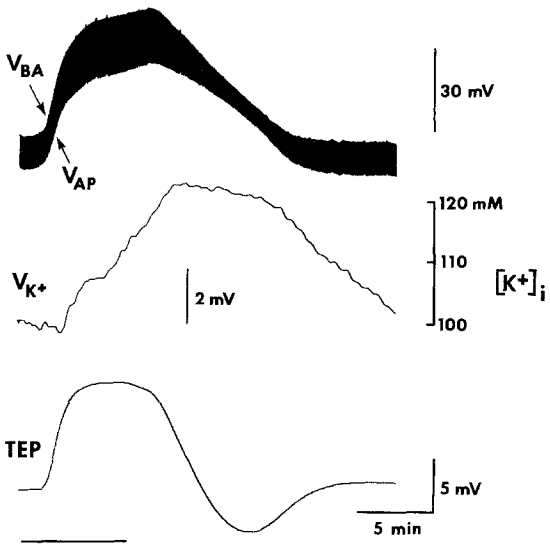

Fig. 9. Measurement of $[K^+]_i$ during a change in basal $[K^+]_o$. The tip of a double-barrel, K^+-specific microelectrode was placed intracellularly; the potentials V_{BA} and V_{AP} were recorded by the reference barrel, and the potential V_{K^+} was recorded differentially between the ion-specific barrel and the reference barrel. The logarithmic scale for $[K^+]_i$ was calculated by using Eq. (3) (*see Materials and Methods*). As a result of perfusion of the basal membrane with high $[K^+]$ Ringer's solution (horizontal bar), $[K^+]_i$ increased from 100 to 122 mM. The changes in membrane potentials were superimposed upon a V_{AP} of -84 mV and a V_{BA} of -73 mV. The initial value of the TEP was $+11.4$ mV

K^+-specific microelectrodes, showed that the basal membrane potential is approximately a measure of the logarithm of basal $[K^+]_o$. After 10 min of perfusing the basal membrane with the high $[K^+]$ solution, the standard Ringer's solution was re-introduced. Following a delay of $\simeq 1$ min, the basal membrane began to hyperpolarize as basal $[K^+]_o$ began to decrease. RPE $[K^+]_i$ continued to increase during this time and reached a maximum several minutes after basal $[K^+]_o$ started to decrease. RPE $[K^+]_i$ remained near this maximum value for $\simeq 4$ min before starting to decrease. It is not surprising that $[K^+]_i$ could increase and remain increased while basal $[K^+]_o$ was decreasing, since basal $[K^+]_o$ was still considerably elevated above its control value during this time. At the end of the time interval illustrated in Fig. 9, $[K^+]_i$ had decreased almost to its original value. The maximum change in V_{K^+} was converted into a change in $[K^+]_i$ by using Eq. (3). This conversion showed that $[K^+]_i$ increased from 100 to 122 mM.

It was possible to record changes in $[K^+]_i$ during perfusion of the basal membrane with high $[K^+]$ solution in 7 cells, as shown in Table 1.

Table 1. Intracellular potassium ion concentration during perfusion of the basal membrane with high $[K^+]$ Ringer's solution[a]

Cell number	Intracellular potassium ion concentration (mM)		
	Resting	Maximum	Change
1	82	106	24
2	102	147	45
3	108	128	20
4	100	122	22
5	138	158	20
6	144	166	22
7	130	141	11
Average:	115 (±8.6)	138 (±7.9)	23 (±3.9)

[a] Data were obtained from 7 cells, using the method shown in Fig. 9. In every case, the intracellular potassium ion concentration, $[K^+]_i$, was obtained from a value of V_{K^+} and Eq. (3). This table contains the initial (resting) value of $[K^+]_i$, the maximum value of $[K^+]_i$ reached after perfusion of the basal membrane with the high $[K^+]$ solution for 8–10 min, and the change in $[K^+]_i$. For each of these variables, the mean value (±SEM) is shown.

In these cells, $[K^+]_i$ averaged 115 ± 8.6 mM, and increased by 23 ± 3.9 mM to 138 ± 7.9 mM as a result of perfusion of the basal membrane with high $[K^+]$ solution for 8–10 min. There was a considerable amount of scatter in the resting value of $[K^+]_i$. Possible reasons for this scatter have been mentioned (*Materials and Methods*). In every cell, however, there was an increase in $[K^+]_i$ produced by the increase in basal $[K^+]_o$. The data are consistent with the hypothesis that an increase in $[K^+]_i$ decreases the electrogenic pump current, which in turn produces the excessive depolarization of the apical membrane.

How Might K^+ Enter the Cell?

The net movement of K^+ into the cell must be accompanied by a simultaneous entry of an anion (Cl^- or HCO_3^-), or by the simultaneous loss of a cation (Na^+ or H^+), in order to preserve electrical neutrality. In skeletal muscle, for example, increasing $[K^+]_o$ at constant $[Cl^-]_o$ upsets a Donnan equilibrium across the cell membrane, and both K^+ and Cl^- (and water) enter the cell in order to produce a new Donnan equilibrium condition (Hodgkin & Horowicz, 1959). If K^+ enters the cell along

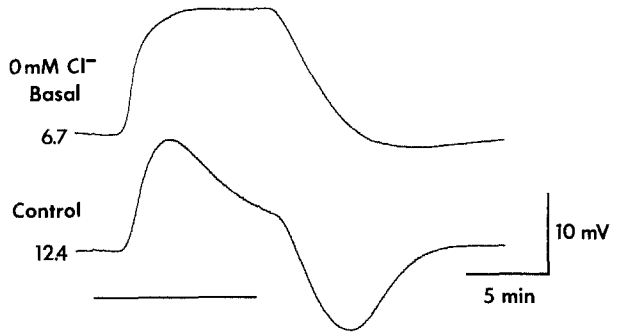

Fig. 10. Effects of 0 mM [Cl^-] (basal) on the K^+-evoked changes in TEP. The basal membrane was perfused with high [K^+] Ringer's solution for the time periods indicated by the horizontal bar. The illustrated responses were recorded sequentially from the same preparation. The number at the left of each trace is the value of the TEP (in mV) at the start of the trace. The basal membrane was perfused with 0 mM [Cl^-] solution for 9 min prior to the start of the upper trace, and with standard Ringer's solution for 13 min prior to the start of the lower trace. For the upper response, the high [K^+] solution used to evoke the change in TEP also contained 0 mM [Cl^-]

with a single *anion*, then removing that anion from the perfusate should prevent the movement of K^+ into the cell, and the decrease in pump current, which seems to be produced by an increase in $[K^+]_i$, should be abolished.

The data in Fig. 10 are from an experiment in which only Cl^- was removed from the solution perfusing the basal membrane. As shown by the upper trace in Fig. 10, this treatment abolished the excessive changes in apical membrane potential (inferred from the absence of an overshoot in the changes in TEP). The effect of 0 mM [Cl^-] was reversible, as shown by the lower trace in Fig. 10, which was recorded 10 min after restoring Cl^- to the solution perfusing the basal membrane. Thus, Cl^- at the basal membrane is necessary for the generation of the "normal" response. It is likely that K^+ can move across the basal membrane only if accompanied by Cl^-.

Even though HCO_3^- was present in the 0 mM [Cl^-] solution, its presence was not sufficient to produce a normal response. In order to confirm that HCO_3^- does not contribute to the generation of the response, only HCO_3^- was removed from the solution perfusing the basal membrane. This treatment had only a very slight effect on the waveform of the K^+-evoked change in TEP (not illustrated). Thus, it is likely that the entry of K^+ into the cell is not accompanied by HCO_3^-.

The excessive apical membrane depolarization was not produced when $[K^+]_o$ was elevated in the apical solution (Fig. 4), perhaps because

the *apical* membrane has no appreciable conductance to Cl^- (Miller & Steinberg, 1977*a*). Consistent with this result, no increase in $[K^+]_i$ was observed when apical $[K^+]_o$ was elevated (not shown).

Discussion

Measurement of $[K^+]_i$

Based upon the changes in basal membrane potential in response to variations in $[K^+]_o$, Miller and Steinberg (1977*a*) concluded that $[K^+]_i$ was between 110 and 150 mM, with the actual value most likely closest to 110 mM. The direct measurement of $[K^+]_i$ with K^+-specific microelectrodes was consistent with this finding. In 14 cells from 9 tissues, the resting level of $[K^+]_i$ was 121 ± 5 mM (mean $\pm$ SEM). In a subset of these cells in which the change in $[K^+]_i$ was measured during perfusion of the basal membrane with high $[K^+]$ Ringer's solution, the resting level of $[K^+]_i$ in 7 cells from 5 tissues was 115 ± 9 mM. The calculated resting level of $[K^+]_i$, however, did show considerable variation, ranging from 82 to 151 mM. (The factors, such as electrode drift, that could have contributed to this large range, were discussed in detail in *Materials and Methods.*)

Inhibition of the Electrogenic Pump by $[K^+]_i$

The excessive depolarization of the apical membrane was prevented by ouabain, cold, or 0 mM $[K^+]_o$ outside the apical membrane, procedures that all inhibit the electrogenic pump on the apical membrane (Miller *et al.*, 1978). Thus, when the electrogenic pump is inhibited the pump current cannot be reduced further by an increase in basal $[K^+]_o$. The direct measurment of $[K^+]_i$, made with K^+-specific microelectrodes, showed that an increase in $[K^+]_i$ of 23 ± 4 mM was associated with the excessive apical depolarization. It is likely, therefore, that the increase in $[K^+]_i$ causes the excessive apical depolarization by reducing the pump current. A similar mechanism has been implicated in red blood cells.

The effects of $[K^+]_i$ on the electrogenic $Na^+ - K^+$ pump of red blood cells have been directly studied using intact human red blood cells (Garay & Garrahan, 1973; Knight & Welt, 1974), reconstituted human red blood cell ghosts (Simons, 1974; Bodemann & Hoffman, 1976), and

inside-out vesicles of human red cells (Blostein & Chu, 1977). Knight and Welt (1974) showed that at fixed levels of $[Na^+]_i$ and $[Na^+]_o$, the efflux of Na^+ through the pump was decreased in high $[K^+]_i$ (e.g., 110 mM) cells and increased in low $[K^+]_i$ (e.g., 69 mM) cells. In general, their data showed that over a wide range of intracellular sodium concentrations (1–20 mM), intracellular potassium was an important determinant of the pump rate. They suggested that $[K^+]_i$ competes with $[Na^+]_i$ for the Na^+ pump sites, and that with higher $[K^+]_i$, the affinity of the pump for Na^+ was reduced, thus diminishing the rate of the pump. This model was also strongly suggested by the data of Garay and Garrahan (1973). They showed, for example, that elevating $[K^+]_i$ (from 39 to 150 mM) reduced the ouabain-sensitive Na^+ efflux over a wide range of internal sodium concentrations (1–30 mM). These results have been corroborated by Simons (1974) and Bodemann and Hoffman (1976) using reconstituted red cell ghosts. In a recent study, Blostein and Chu (1977) studied the side-specific interactions of K^+ with inside-out membrane vesicles of human red blood cells. They found that intracellular K^+ inhibited the activation of the $(Na^+ - K^+)$-ATPase, consistent with it being a competitive inhibitor of the $Na^+ - K^+$ pump.

In the RPE, it is possible that elevating basal $[K^+]_o$ causes a reduction in $[Na^+]_i$. This reduction could occur by a movement of KCl across the basal membrane that is osmotically followed by water. It is unlikely, however, that any such reduction in $[Na^+]_i$ is the *sole* cause of the pump inhibition, since the experiments of Knight and Welt (1974) on red blood cells showed that potassium exerts its strongest inhibitory effect when cell sodium is low or normal.

The present experiments show that Cl^- is the counterion for K^+, and thus it is likely that an increase in $[Cl^-]_i$ accompanies the increase in $[K^+]_i$. The inhibition of the electrogenic sodium pump, therefore, could be caused by the increase in $[Cl^-]_i$. To our knowledge, however, there is no evidence to support the idea that an increase in $[Cl^-]_i$ can directly inhibit either the $(Na^+ - K^+)$-ATPase or the $Na^+ - K^+$ pump. Alternatively, an increase in $[Cl^-]_i$ could stimulate another electrogenic mechanism; for example, a $Cl^- - HCO_3^-$ exchange pump. This pump would have to be located on the apical membrane and be sensitive to ouabain. It has been shown that Cl^- is actively transported across the RPE, but it is not known whether the active transport mechanism is located on the apical or basal membrane (Miller & Steinberg, 1977*b*).

There is evidence in several other epithelia that anion pumps may be driven by a $(Na^+ - K^+)$-ATPase or coupled to the $Na^+ - K^+$ pump and,

therefore, sensitive to ouabain (Wright, 1978; Watlington & Jessee, 1975; Watlington, Jessee & Baldwin, 1977). In the RPE, however, it is very unlikely that the chloride transport system is driven by a $(Na^+ - K^+)$-ATPase or directly coupled to the $Na^+ - K^+$ pump, since the substances that inhibit one system do not inhibit the other, and vice versa (Miller & Steinberg, 1977*b*). It is possible that the movement of HCO_3^- across the apical membrane is linked to the $Na^+ - K^+$ pump, but this linkage is not likely to be direct, since changes in $[HCO_3^-]_o$ do not affect the unidirectional fluxes of Na^{22} (S.S. Miller & R.H. Steinberg, *unpublished*).

Potassium Ion and Pigment Epithelial Transport

The retinal pigment epithelium transports ions and nutrients across its cell membranes, and thus participates in controlling the composition of the extracellular space surrounding the photoreceptors. There is evidence that potassium ion plays an important part in the regulation of this transport. Previous work showed that photic stimulation of the retina produces a decrease in $[K^+]_o$ outside the apical membrane, which alters the apical membrane potential (Oakley & Green, 1976; Oakley *et al.*, 1977; Oakley, 1977). Recent work demonstrated that such changes in extracellular K^+ can affect the magnitude of active metabolite and ion transport across the RPE (S.S. Miller & R.H. Steinberg, *unpublished*). Since the present work demonstrates a correlation between changes in intracellular K^+ and the electrogenic $Na^+ - K^+$ pump of the apical membrane, it is now clear that K^+ can alter transport from both the inside and outside of the apical membrane. It remains to be shown whether or not changes in intracellular K^+ are involved in the responses to photic stimulation.

We would like to thank Mr. Herman B. Chibnik for technical assistance. This work was supported in part by National Institutes of Health postdoctoral fellowship EY-05048 to B. Oakley and National Institutes of Health grant EY-01429 to R.H. Steinberg.

Appendix

In this appendix, the changes in membrane potential associated with perfusing either the apical or basal membranes with high $[K^+]$ Ringer's solution are analyzed by a quantitative method. The results of this

analysis indicate that a passive electrical analogue of the retinal pigment epithelium (Miller & Steinberg, 1977*a*) is inadequate to explain the changes in apical membrane potential observed during long lasting (>3 min) increases in basal $[K^+]_o$.

The passive model of the RPE is shown in Fig. 1 of the previous paper (Miller *et al.*, 1978). For the present purpose, the contribution of the electrogenic pump current to the membrane potentials is assumed to be constant. Since both the apical and basal membranes have an appreciable potassium conductance (Miller & Steinberg, 1977*a*), both membrane batteries (V'_{AP} and V'_{BA}, respectively) contain a term involving the potassium equilibrium potential. Thus, an increase in $[K^+]_o$ outside either membrane will cause that membrane to depolarize via a direct effect on the membrane battery. A decrease in the magnitude of one membrane battery will, in turn, change the shunt current in a direction so that the opposite membrane will also depolarize.

Based upon the passive model of the RPE, it can be shown that for all changes in V'_{AP}

$$R = \frac{\Delta V_{BA}}{\Delta V_{AP}} = \frac{R_{BA}}{R_{BA} + R_S} \leqq 1 \tag{A1}$$

and that for all changes in V'_{BA}

$$R^* = \frac{\Delta V_{AP}}{\Delta V_{BA}} = \frac{R_{AP}}{R_{AP} + R_S} \leqq 1 \tag{A2}$$

where R and R^* are dimensionless ratios and it is assumed that all the resistances remain constant. These equations predict that the change in membrane potential will be larger across the membrane where $[K^+]_o$ was changed than across the opposite membrane. It has been shown that the resistances do not remain constant when $[K^+]_o$ is altered (Miller & Steinberg, 1977*a*), but even if all the resistances change by a factor of two, which is a greater change than has been observed, the dimensionless ratios R and R^* will still be less than 1. The predictions stated in Eqs. (A1) and (A2) can be tested directly.

For an increase in $[K^+]_o$ outside the apical membrane, the model predicted that the ratio R (equal to $\Delta V_{BA}/\Delta V_{AP}$) should always be less than 1. This prediction was tested in Fig. 11. When $[K^+]_o$ was increased outside the apical membrane, V_{AP} depolarized, and due to passive shunting, V_{BA} also depolarized. The change in V_{BA}, however, was less than the change in V_{AP}, so that these membrane potential changes caused

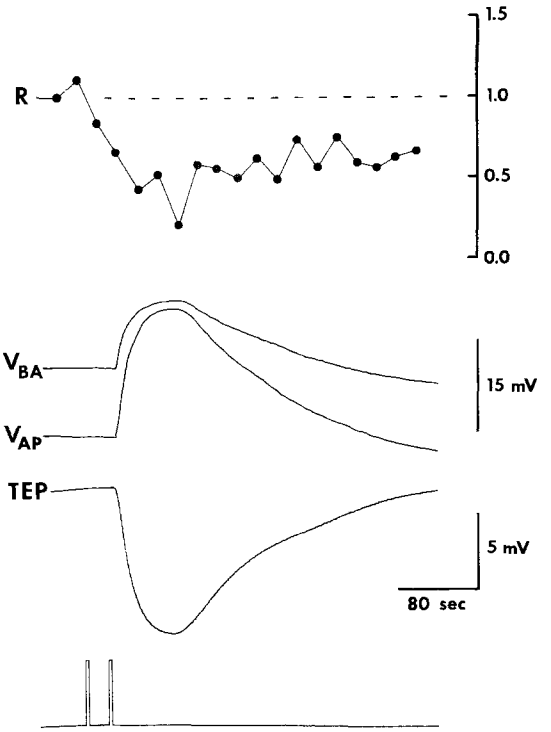

Fig. 11. Changes in V_{BA}, V_{AP}, and TEP as a result of perfusion of the *apical* membrane with high $[K^+]$ Ringer's solution. The high $[K^+]$ solution flowed into the apical chamber during the time interval between the two pulses on the lower trace. The responses were superimposed upon a V_{AP} of -88 mV and a TEP of 10.6 mV. The illustrated response was divided into 20 equally-spaced time intervals, and for each interval, a value of R (equal to $\Delta V_{BA}/\Delta V_{AP}$; *see* text) was calculated by the computer and plotted at the top of the illustration

the TEP to decrease. By using a computer (*see Materials and Methods*), it was possible to quantify the value of the ratio R at 20 equally spaced times. The calculated values of R are plotted above the membrane potential changes. During the time when the membrane potentials were changing, the value of R was always less than 1. This result is consistent with the model in Fig. 1 of Miller *et al.*, (1978), assuming that there are no changes in electrogenic pump current.

For an increase in $[K^+]_o$ outside the basal membrane, a much different result was observed, as shown in Fig. 12. As predicted, the increase in $[K^+]_o$ initially depolarized the basal membrane, and due to passive shunting, the apical membrane was also depolarized. The value of R^* (equal to $\Delta V_{AP}/\Delta V_{BA}$) was calculated by the computer at 20 times, and plotted above the changes in membrane potentials. During two different

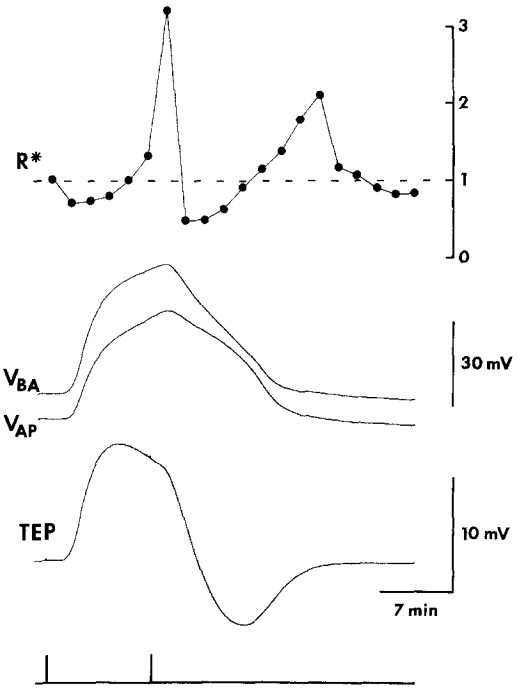

Fig. 12. Changes in V_{BA}, V_{AP}, and TEP as a result of perfusion of the *basal* membrane with high $[K^+]$ Ringer's solution. The high $[K^+]$ solution flowed into the basal chamber during the time interval between the two pulses on the lower trace. The responses were superimposed upon a V_{AP} of -84 mV and a TEP of $+12.6$ mV. The illustrated response was divided into 20 equally-spaced time intervals, and for each interval, a value of R^* (equal to $\Delta V_{AP}/\Delta V_{BA}$; *see* text) was calculated by the computer and plotted at the top of the illustration

intervals of the response, the calculated values of R^* were greater than 1, indicating that the apical membrane potential changed by a greater amount than did the basal membrane potential. During one interval, the depolarization of the apical membrane was >3 times larger than the depolarization of the basal membrane, and during another interval, the hyperpolarization of the apical membrane was >2 times larger than the hyperpolarization of the basal membrane, as indicated by the values of R^*. These results *cannot* be explained by the passive model, unless extremely large variations in the membrane and shunt resistances occur. Such resistance changes have not been observed (Miller & Steinberg, 1977*a*). When the model is modified to include a variable contribution to the apical membrane potential from an electrogenic pump, then the responses of the apical membrane can be explained in terms of variations in the pump current.

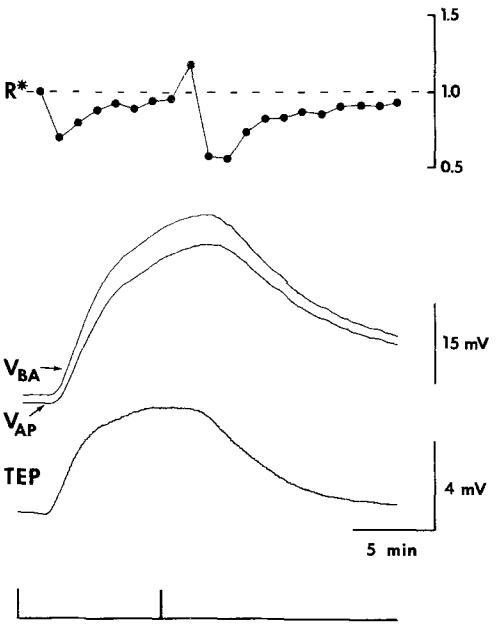

Fig. 13. Changes in V_{BA}, V_{AP}, and TEP in a ouabain-treated preparation as a result of perfusion of the *basal* membrane with high $[K^+]$ Ringer's solution. The apical membrane was perfused with ouabain (10^{-4} M) Ringer's solution for 18 min prior to (and during) the illustrated time period. The basal membrane was perfused with high $[K^+]$ solution during the time interval between the two pulses on the bottom trace. The responses were superimposed upon a V_{AP} of -65 mV and a TEP of $+3.1$ mV. The illustrated time period was divided into 20 equally-spaced time intervals, and for each interval, a value of R^* (equal to $\Delta V_{AP}/\Delta V_{BA}$; *see* text) was calculated by the computer and plotted at the top of the illustration

If ouabain is applied to the apical membrane, then the electrogenic pump current should be abolished, and the passive equivalent circuit of the RPE should become appropriate. In Fig. 13, effects of increasing $[K^+]_o$ outside the basal membrane are shown that were recorded from a tissue that was treated with ouabain (applied only to the apical membrane). Again, the value of the ratio R^* (equal to $\Delta V_{AP}/\Delta V_{BA}$) was computed at 20 times, and plotted above the changes in membrane potentials. As predicted by the model, the value of the ratio R^* was less than 1 (except during one interval when neither V_{AP} nor V_{BA} changed appreciably, leading to R^* equal to 0/0). Thus, it seems that in Fig. 12, variations in the electrogenic pump current were responsible for the departure of the changes in membrane potential from those predicted by the model.

In conclusion, a quantitative method has been used to characterize the changes in apical and basal membrane potentials caused by increases in $[K^+]_o$ outside of either membrane. With this method, it was possible to show that the equivalent circuit, without a variable contribution from the electrogenic pump, could not account for the changes in apical membrane potential resulting from changes in basal $[K^+]_o$. When the apical membrane was treated with ouabain, however, and the contribution of the electrogenic pump was eliminated, the membrane potential changes were consistent with the passive electrical analogue (Miller & Steinberg, 1977*a*).

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