

Ba²⁺ Unmasks K⁺ Modulation of the Na⁺-K⁺ Pump in the Frog Retinal Pigment Epithelium

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ABSTRACT This paper presents electrophysiological evidence that small changes in $[K^+]_o$ modulate the activity of the Na⁺-K⁺ pump on the apical membrane of the frog retinal pigment epithelium (RPE). This membrane also has a large relative K⁺ conductance so that lowering $[K^+]_o$ hyperpolarizes it and therefore increases the transepithelial potential (TEP). Ba²⁺, a K⁺ channel blocker, eliminated these normal K⁺-evoked responses; in their place, lowering $[K^+]_o$ evoked an apical depolarization and TEP decrease that were blocked by apical ouabain or strophanthidin. These data indicate that Ba²⁺ blocked the major K⁺ conductance(s) of the RPE apical membrane and unmasked a slowing of the normally hyperpolarizing electrogenic Na⁺-K⁺ pump caused by lowering $[K^+]_o$. Evidence is also presented that $[K^+]_o$ modulates the pump in the isolated RPE under physiological conditions (i.e., without Ba²⁺). In the intact retina, light decreases subretinal $[K^+]_o$ and produces the vitreal-positive c-wave of the electroretinogram (ERG) that originates primarily in the RPE from a hyperpolarization of the apical membrane and TEP increase. When Ba²⁺ was present in the retinal perfusate, the apical membrane depolarized in response to light and the TEP decreased so that the ERG c-wave inverted. The retinal component of the c-wave, slow PIII, was abolished by Ba²⁺. The effects of Ba²⁺ were completely reversible. We conclude that Ba²⁺ unmasks a slowing of the RPE Na⁺-K⁺ pump by the light-evoked decrease in $[K^+]_o$. Such a response would reduce the amplitude of the normal ERG c-wave.

INTRODUCTION

The retinal pigment epithelium (RPE) is a single-cell layer that separates the neural retina from the choroidal blood supply. The apical membrane of the RPE faces the photoreceptors across the subretinal space, while the basal (basolateral) membrane faces the choroid. One of the many functions of the RPE is transport via an Na⁺-K⁺ ATPase that has been localized to the apical membrane

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(Miller et al., 1978; Bok and Filerman, 1979; Bok, 1982; Ostwald and Steinberg, 1981). Tracer studies across the isolated RPE-choroid preparation have shown that transport via the Na⁺-K⁺ pump is a function of the concentration of K⁺ outside the apical membrane, [K⁺]_o (Miller and Steinberg, 1977*b*, 1982; Steinberg and Miller, 1979). In this paper, we present an electrophysiological study of the pump and its modulation by changes in [K⁺]_o in both the isolated RPE-choroid and in the intact retina (neural retina-RPE-choroid).

Previous studies have shown that the RPE Na⁺-K⁺ pump is electrogenic and that it normally contributes ~10 mV to the apical membrane potential (Miller et al., 1978). Although it was also shown that this pump is inhibited by 0 [K⁺]_o (Oakley et al., 1978), until now it has not been possible to study the effects of changes in [K⁺]_o on the electrogenic contribution of the pump because such effects are normally masked by a larger passive response of the apical membrane evoked by changes in [K⁺]_o. This passive response is a consequence of the high relative K⁺ conductance of the RPE apical membrane. In this study, we examine apical responses from the frog RPE evoked by changes in [K⁺]_o after blocking the apical K⁺ conductance, g_{K^+} , with Ba²⁺. In the presence of Ba²⁺, the modulation of the pump by [K⁺]_o was unmasked.

METHODS

Preparation and Solution

Two retinal preparations of bullfrog, *Rana catesbeiana*, were used. The chamber design and the techniques for dissecting and mounting the tissue were identical to those used by Miller and Steinberg (1977*a*). In brief, a tissue consisting of neural retina, RPE, and choroid was dissected after first light-adapting the animal to minimize retinal detachment. The animal was decapitated, the eye was enucleated, and the posterior half of the eye was submerged in perfusate. The retina-RPE-choroid was dissected free from the sclera and mounted between two Lucite plates. The preparation was then dark-adapted until the ERG c-wave reached a stable amplitude. To isolate an RPE-choroid preparation, the animal was first dark-adapted to facilitate removal of the neural retina. The remaining RPE-choroid was dissected free from the sclera and mounted.

In both preparations, the two sides of the tissue, retinal (or apical) and choroidal (or basal), were continuously superfused at 2–5 ml/min by a gravity feed system from two large reservoirs. The area of the tissue exposed to the perfusate was 0.07 cm². The control perfusate was a modified Ringer's solution having the following composition (mM): 82.5 NaCl, 27.5 NaHCO₃, 2.0 KCl, 1.0 MgCl₂, 1.8 CaCl₂, 10.0 glucose, bubbled with 95% O₂ and 5% CO₂, pH 7.4. The apical and basal perfusates could be independently switched to test solutions with altered KCl in the presence or absence of BaCl₂. The NaCl concentration was adjusted to keep osmolarity constant when changes of >3.0 mM KCl were made. Light stimuli were presented as described previously (Griff and Steinberg, 1982); the unattenuated white light stimulus delivered 3.7 mW/cm².

Electrodes

Conventional microelectrodes were made from 1.0-mm tubing (Omega Dot Glass Co. of America, Millville, NJ), filled with 5 M K⁺ acetate, and beveled to a resistance of 50–100 MΩ. Unity gain preamplifiers (model 1090, Winston Electronics, San Francisco, CA) with input resistances of 10¹⁴ Ω were used to measure microelectrode potentials.

K^+ -selective microelectrodes were constructed from double-barreled glass tubing (fused side by side, each barrel 1.0 mm), as described in detail by Oakley (1977). One barrel was filled with a K^+ exchanger solution (477317, Corning Medical Products Div., Medfield, MA) and the second (reference) barrel with 5 M LiCl. K^+ microelectrodes were beveled until the reference barrel had a resistance of 40–80 $M\Omega$; the tip size was $\sim 1 \mu m$. The potential, V_{K^+} , between the K^+ barrel and the reference barrel was proportional to the logarithm of $[K^+]_o$ (Oakley, 1977). Each K^+ -selective electrode was calibrated before an experiment in solutions of varying $[K^+]_o$ but constant $[K^+]_o + [Na^+]_o$. The presence of 0.2–1.0 mM $BaCl_2$ in the test solutions had negligible effects on the responses of these

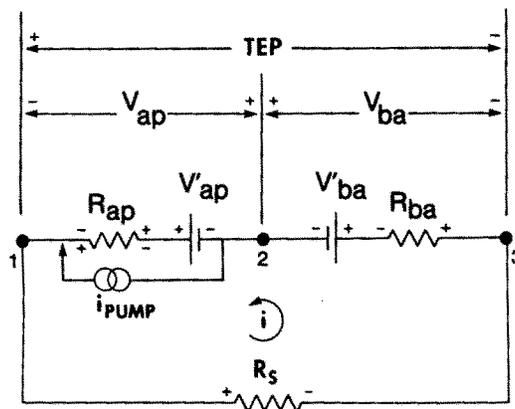


FIGURE 1. Equivalent circuit for the RPE. The apical membrane is represented by a resistor (R_{ap}) in series with a battery (V'_{ap}). Similarly, the basal membrane is represented by a resistor (R_{ba}) in series with a battery (V'_{ba}). The membrane resistances are shunted by a resistor (R_s). Because of the difference between V'_{ap} and V'_{ba} , a steady current (i) flows through the circuit. The electrogenic pump on the apical membrane is modeled as a current source that produces a constant current (i_{pump}). As a result of current flow across the apical and basal membrane resistances, the potentials recorded across them (V_{ap} and V_{ba} , respectively) differ from the batteries (V'_{ap} and V'_{ba}). The steady potential across the isolated RPE-choroid is called the transepithelial potential (TEP). The potentials V_{ap} , V_{ba} , and TEP are labeled with the polarity with which they were recorded. In all figures, these potentials are displayed with positive polarity upward. Extracellular electrodes were placed in the apical perfusate (position 1) and in the basal perfusate (position 3); a microelectrode was placed intracellularly (position 2).

K^+ -selective electrodes. Absolute K^+ concentrations were determined from the calibration curve for the electrode referenced to the known $[K^+]_o$ in the bathing solution (usually 2.0 mM) as described by Oakley (1977).

Equivalent Circuit

Fig. 1 summarizes the equivalent circuit of the RPE. In this circuit, the apical membrane is represented as a resistance, R_{ap} , in series with a battery, V'_{ap} . Similarly, the basal membrane is modeled as a resistance, R_{ba} , in series with a battery, V'_{ba} . The two RPE membranes are connected by a resistor, R_s , which represents the parallel combination of the paracellular resistance across the intercellular junctional complexes and a resistive

pathway around the edge of the tissue. In control conditions, a steady current, i , flows through the circuit because V'_{ap} is greater in magnitude than V_{ba} . This current hyperpolarizes the basal membrane and depolarizes the apical membrane (Miller and Steinberg, 1977a). Thus, the potentials recorded by an intracellular microelectrode, V_{ap} and V_{ba} , differ in absolute magnitude from the membrane batteries. A change in one of the membrane batteries (e.g., $\Delta V'_{ap}$) will also change the current, i , so that both membrane potentials will change. The change in membrane potential will be greater in magnitude for the membrane where the potential (battery change) originates; the smaller change at the other membrane is a passive "shunted" response. The transepithelial potential (TEP) is the difference between V_{ba} and V_{ap} . The derivation of the equations that describe these potential changes has been presented previously (Miller and Steinberg, 1977a; Linsenmeier and Steinberg, 1983). In summary, for a potential generated at the apical membrane,

$$\Delta V_{ap} = \Delta V'_{ap} \left(\frac{R_{ba} + R_s}{R_{ap} + R_{ba} + R_s} \right), \quad (1)$$

and

$$\Delta V_{ba} = \Delta V'_{ap} \left(\frac{R_{ba}}{R_{ap} + R_{ba} + R_s} \right). \quad (2)$$

It will be shown that the apical membrane also contains a separate source of steady current, i_{pump} , which results from a hyperpolarizing electrogenic pump. Part of the pump current flows through R_{ap} , hyperpolarizing the apical membrane, and part of it flows through R_s and then through R_{ba} , hyperpolarizing the basal membrane. The major fraction of the pump current flows across the apical membrane since R_{ap} is smaller than the sum of R_s and R_{ba} (Miller and Steinberg, 1977a). As shown by Miller et al. (1978), the pump current hyperpolarizes the apical and basal membranes by

$$\Delta V_{ap} = i_{\text{pump}} \frac{R_{ap}(R_{ba} + R_s)}{R_{ap} + R_{ba} + R_s}; \quad (3)$$

$$\Delta V_{ba} = i_{\text{pump}} \frac{(R_{ap})(R_{ba})}{R_{ap} + R_{ba} + R_s}. \quad (4)$$

Recording Configuration

The placement of electrodes and recording configuration have been previously described (Griff and Steinberg, 1984; Oakley, 1977). In brief, the trans-tissue potential (TEP for the isolated RPE-choroid preparation; ERG for the retina-RPE-choroid) was measured differentially between two calomel electrodes that were connected to the retinal (or apical) and basal baths by a pair of agar-Ringer's bridges. To record the TEP in the retina-RPE-choroid preparation, a microelectrode was positioned in the subretinal space (Fig. 1, position 1). The transretinal potential was simultaneously recorded between the same microelectrode and the calomel in the retinal bath (not shown). In both preparations, the microelectrode could also be positioned intracellularly in the RPE (position 2). In the isolated RPE-choroid, V_{ap} and V_{ba} were recorded simultaneously by measuring differentially between the intracellular microelectrode and the apical (position 1) and basal (position 3) baths, respectively. For the retina-RPE-choroid, we recorded simultaneously between the intracellular microelectrode and the retinal and basal baths. V_{ba} is the basal membrane potential; $V_{ap} + V_R$ is the sum of the apical membrane potential and the extracellular voltage across the retina (V_R).

Current pulses (1.0 μ A, 1.0 s) were injected across the isolated RPE preparation through two silver-chlorided wires connected to each bath by a second pair of bridges. A resistance and a resistance ratio were determined from the appropriate current-induced voltage (iR) drops. The transepithelial resistance, R_t , is equal to $(R_s)(R_{ap} + R_{ba})/(R_{ap} + R_{ba} + R_s)$ and is proportional to the iR drop across the RPE. The ratio of R_{ap} to R_{ba} is called a and is proportional to the ratio of the iR drops across the apical and basal membranes. For the retina-RPE-choroid preparation, current was injected across the tissue so that R_t and a were contaminated by the iR drop across the neural retina.

The tissues used in these studies met the same minimal criteria for TEP, R_t , and RPE membrane potentials as in previous studies of frog RPE. The mean TEP in control Ringer's for the isolated RPE-choroid preparation was 10.9 ± 3.0 mV (SD) and the mean transepithelial resistance, R_t , was 3.6 ± 0.7 k Ω (SD; $n = 32$ tissues). Intracellular recordings were made from 44 RPE cells with a mean apical resting potential (V_{ap}) of 87 ± 6 mV (SD).

RESULTS

The results are presented in two sections. First, we use a preparation of isolated RPE-choroid, where there is direct access to the apical membrane, to demonstrate that small changes in $[K^+]_o$ modulate the RPE Na^+K^+ pump. Second, we show that a similar modulation of the RPE Na^+K^+ pump also occurs in the intact retina (neural retina-RPE-choroid) in response to light-evoked changes in sub-retinal $[K^+]_o$.

Modulation of the Na^+K^+ Pump in the Isolated RPE-Choroid

The RPE apical membrane has a large relative K^+ conductance so that its membrane potential is a function of $[K^+]_o$. Fig. 2A shows responses from an isolated RPE-choroid evoked by decreasing $[K^+]_o$ from 3.0 to 1.0 mM. The TEP increased because the apical membrane hyperpolarized relative to a smaller basal hyperpolarization. The apical response is expected since the K^+ equilibrium potential (E_K) becomes more negative. The smaller basal response resulted from current flowing through the paracellular shunt and across the basal membrane resistance (see Methods, Fig. 1 and Eq. 2).

In this paper, we examine RPE responses after blocking an apical K^+ conductance with Ba^{2+} . Fig. 2B shows the effects on RPE membrane potentials of switching the apical perfusate to one containing 0.2 mM Ba^{2+} . When the test solution reached the tissue, the apical membrane depolarized relative to the basal membrane so that TEP decreased. As indicated below (Fig. 3), Ba^{2+} can eliminate the apical response to a change in $[K^+]_o$, which suggests that it blocks the major K^+ conductance(s) of the apical membrane. A depolarization of the apical membrane is expected when the K^+ conductance is blocked since the apical membrane potential is now set by other ions to which this membrane is permeable (Miller and Steinberg, 1977a). A reduction in the coupling ratio of the Na^+K^+ pump may also contribute to the apical depolarization (Walter and Sillman, 1984). The smaller basal depolarization is presumably a "shunted" response and therefore should be a constant percent of the apical depolarization. Thus, when the apical and basal responses were scaled to the same amplitude, the two responses superimposed exactly (not shown).

To estimate the magnitude of the expected increase in apical membrane resistance, R_{ap} , owing to Ba^{2+} , pulses of current were injected across the tissue and the appropriate voltage drops (iR) were measured. The iR drop across the tissue is proportional to R_t and the ratio of iR drops across the apical and basal membranes, a , to R_{ap}/R_{ba} . If only R_{ap} increased in Ba^{2+} , then R_t and a should

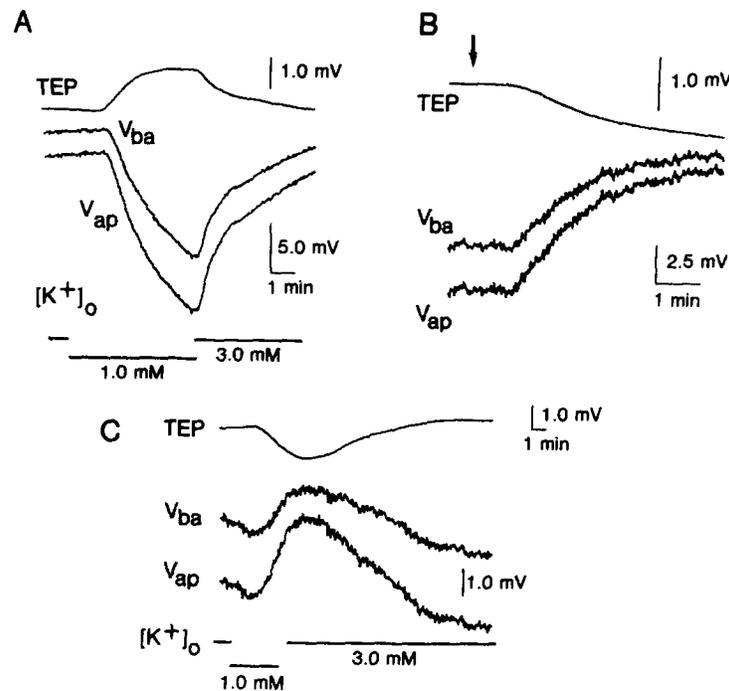


FIGURE 2. Comparison of RPE responses in the presence and absence of Ba^{2+} . TEP was recorded differentially between the apical and basal perfusates. Simultaneously, the apical membrane potential (V_{ap}) was recorded differentially between an intracellular microelectrode and the apical perfusate; the basal membrane (V_{ba}) was recorded between the microelectrode and the basal perfusate. In this and subsequent figures, the timing of $[K^+]_o$ changes in the apical perfusate is shown underneath the traces. (A) Control responses evoked by decreasing $[K^+]_o$ from 3.0 to 1.0 and then back to 3.0 mM. When $[K^+]_o$ decreased, the TEP increased because V_{ap} hyperpolarized relative to V_{ba} . (B) The effects of changing the apical perfusate to one containing 0.2 mM $BaCl_2$ (arrow). TEP decreased because V_{ap} depolarized relative to V_{ba} . (C) Responses evoked by decreasing $[K^+]_o$ in the presence of Ba^{2+} ; TEP decreased because V_{ap} depolarized relative to V_{ba} .

both increase. However, the resistance changes produced by adding 0.2 mM $BaCl_2$ to the apical perfusate were more complicated, and these findings are described in detail in the Appendix. In summary, Ba^{2+} increased R_{ap} and decreased R_{ba} so that the ratio a increased, while R_t remained unchanged or decreased. The increase in R_{ap} is consistent with a block of an apical K^+

conductance by Ba^{2+} . Evidence presented in Fig. 14 (see Appendix) suggests that the decrease in R_{ba} is due to an increase in a basal K^+ conductance but the exact mechanism of this effect remains unknown. Since the basal membrane is at or near the K^+ equilibrium potential (Miller and Steinberg, 1977a), one would not expect a change in basal K^+ conductance to directly alter the basal membrane potential.

An examination of the responses of the apical membrane to changes in $[K^+]_o$ in the presence of Ba^{2+} provided further evidence that Ba^{2+} blocked an apical K^+ conductance. These experiments also provided the first evidence that Ba^{2+} unmasked a response that represented modulation of the apical Na^+-K^+ pump. Fig. 2C shows RPE responses to a decrease in $[K^+]_o$ from 3.0 to 1.0 mM in 1.0 mM $BaCl_2$. Instead of a TEP increase generated by an apical hyperpolarization as in Fig. 2A, lowering $[K^+]_o$ in the presence of Ba^{2+} decreased TEP. The intracellular recordings showed that the apical membrane depolarized relative to the basal membrane so that an apical depolarization generated the TEP decrease. For a decrease from 3.0 to 1.0 mM $[K^+]_o$, the maximum absolute depolarization of the apical membrane was 2.7 mV.

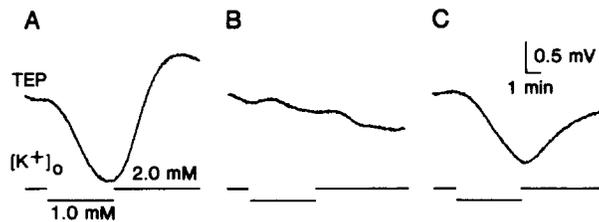


FIGURE 3. Effects of strophanthidin and Ba^{2+} on RPE responses. (A) In the presence of 0.2 mM Ba^{2+} , lowering $[K^+]_o$ from 2.0 to 1.0 decreases TEP; when $[K^+]_o$ is increased back to 2.0 mM, TEP recovers. Strophanthidin (10^{-5} M) was added to the apical perfusate, and the $[K^+]_o$ change was repeated in the continued presence of Ba^{2+} and strophanthidin (B). Strophanthidin abolished the TEP response to the $[K^+]_o$ change. When the strophanthidin was washed out, the response evoked by a $[K^+]_o$ change in the presence of Ba^{2+} recovered (C).

Decreasing $[K^+]_o$ Modulates the Pump

The results shown in Fig. 2C suggested the hypothesis that Ba^{2+} , by blocking an apical K^+ conductance, prevented the apical hyperpolarization caused by the increase in E_K and unmasked a slowing of the electrogenic Na^+-K^+ pump located on the apical membrane. Since the pump normally hyperpolarizes the apical membrane by ~ 10 mV, slowing the pump would depolarize the membrane and decrease TEP. Since pump current also flows across the paracellular shunt and basal membrane resistance, slowing of the pump would also depolarize the basal membrane (see Methods, Fig. 1 and Eqs. 3 and 4).

To test this hypothesis, we examined the effects of the pump inhibitors ouabain and strophanthidin on RPE responses evoked by changing $[K^+]_o$ in the presence of Ba^{2+} . Fig. 3 shows TEP responses before (A), during (B), and after (C) addition

of strophanthidin to the apical perfusate. In Fig. 3A, lowering $[K^+]_o$ from 2.0 to 1.0 mM in the presence of 0.2 mM Ba^{2+} decreased TEP; with the increase of $[K^+]_o$ back to 2.0 mM, TEP overshoot the baseline and then slowly returned. This response resembles the one shown in Fig. 2C. Next, 10^{-5} M strophanthidin was added to the apical perfusate to poison the Na^+K^+ pump. As expected, TEP decreased rapidly (not shown) as a result of an apical depolarization caused by elimination of the pump's electrogenic contribution to the apical membrane potential (Miller et al., 1978). After the TEP had stabilized (15 min), $[K^+]_o$ was again switched from 2.0 to 1.0 mM, now in the presence of both Ba^{2+} and strophanthidin. As shown in Fig. 3B, the response was abolished. Intracellular recordings (not shown) demonstrated the absence of either an apical or basal membrane response. After washout of the strophanthidin (~ 120 min), the TEP response to the decrease in $[K^+]_o$ in Ba^{2+} partially recovered. Similar results were obtained using 10^{-4} M ouabain but the effects were not reversible.

The results of Fig. 3 support our hypothesis that Ba^{2+} blocked an apical K^+ conductance and unmasked a modulation of the Na^+K^+ pump. Since ouabain and/or strophanthidin blocked the TEP decrease evoked by lowering $[K^+]_o$ in the presence of Ba^{2+} , we concluded that this TEP decrease was generated by the apical Na^+K^+ pump. Since the combined treatment of Ba^{2+} and strophanthidin (or ouabain) blocked all responses evoked by changing $[K^+]_o$, we conclude that the major apical K^+ conductance(s) was completely blocked by Ba^{2+} . Ba^{2+} and K^+ compete for the K^+ channel (e.g., Eaton and Brodwick, 1980; Armstrong and Taylor, 1980), so that the concentration of Ba^{2+} needed to block the responses to changing $[K^+]_o$ depended on the initial K^+ concentration. We found that 0.2 mM Ba^{2+} was sufficient to block responses when $[K^+]_o$ was <2.0 mM and 1.0 mM Ba^{2+} was sufficient to block responses when $[K^+]_o$ was <5.0 mM.

Is the Pump Modulated by $[K^+]_o$ in the Absence of Ba^{2+} (Normal Condition)?

The Ba^{2+} results suggested that a $[K^+]_o$ decrease may produce two apical potentials of opposite sign under normal physiological conditions (i.e., in the absence of Ba^{2+}): an apical hyperpolarization mediated by an apical K^+ conductance, and an apical depolarization mediated by the Na^+K^+ pump. These two apical potentials presumably sum algebraically in the normal response; since the hyperpolarizing component is larger, it dominates and generates the normal increase in TEP. If the pump was poisoned to block the depolarizing component, then the apical hyperpolarization should appear larger by an amount equal in magnitude to the contribution of the pump in the normal response.

Stable intracellular measurements were obtained in five experiments in which RPE responses produced by decreasing $[K^+]_o$ could be compared in the presence and absence of ouabain. In each experiment, at least two $[K^+]_o$ changes were made before adding ouabain to establish that the amplitude of the response was constant. The $[K^+]_o$ change in the presence of ouabain was made at least 10 min after the introduction of ouabain.

Fig. 4 compares RPE responses evoked by a decrease in $[K^+]_o$ from one experiment before (A) and after (B) poisoning the pump by adding ouabain to the apical perfusate; the intracellular recordings were all from the same RPE

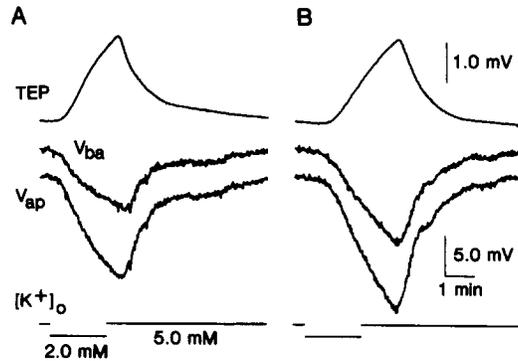


FIGURE 4. Effects of ouabain on $[K^+]_o$ -evoked RPE responses. TEP, V_{ap} , and V_{ba} were recorded as in Fig. 2. (A) Control responses evoked by decreasing $[K^+]_o$ from 5.0 to 2.0 and back to 5.0 mM. Ouabain (10^{-4} M) was then added continuously to the apical perfusate. (B) Responses evoked by the same $[K^+]_o$ change but in the presence of ouabain; same cell as in A. In ouabain, decreasing $[K^+]_o$ evoked larger responses than in control.

cell. The responses evoked by lowering $[K^+]_o$ were larger in the presence of ouabain than in the control. For a change from 5.0 to 2.0 mM $[K^+]_o$, the apical hyperpolarization increased by a maximum of 3.7 mV in the presence of ouabain, as shown in Fig. 4. In four of the five experiments, the apical hyperpolarization increased in ouabain (Table I). For a change in $[K^+]_o$ from 3.0 to 1.0 mM, the maximum increase was 2.2 mV. In most cases, the TEP response in ouabain decreased, although the apical and basal (not shown) responses both increased. This could occur if, in spite of the increase in the apical membrane response caused by blockage of the pump, more of the apical response was shunted to the basal membrane, presumably because of a decrease in paracellular resistance, R_s .

TABLE I
Effects of Ouabain on $[K^+]_o$ -evoked RPE Responses

$[K^+]_o$ change mM	Apical response		TEP response	
	Control mV	Ouabain mV	Control mV	Ouabain mV
5.0 to 2.0	11.7	15.4	2.2	2.2
5.0 to 2.0	18.0	20.4	1.7	1.0
3.0 to 1.0	15.2	16.5	1.3	1.6
3.0 to 1.0	18.0	20.2	1.7	1.0
3.0 to 1.0	20.6	15.9	4.0	2.4

Stable intracellular measurements were obtained in five experiments in which RPE responses produced by a change in $[K^+]_o$ could be compared in the presence and absence (control) of 10^{-4} M ouabain. In each experiment, at least two $[K^+]_o$ changes were made in control perfusate to establish that the amplitude of the response was constant. The $[K^+]_o$ change in the presence of ouabain was made at least 10 min after the ouabain was introduced. Ouabain itself produced a steady apical depolarization and TEP decrease; these were digitally subtracted from the $[K^+]_o$ -evoked responses in ouabain to determine their amplitudes.

Consistent with this hypothesis, R_t decreased in ouabain. Moreover, the resistance changes in ouabain are in the direction to underestimate the contribution of the pump to the apical response.¹

Properties of the $[K^+]_o$ -evoked Pump Response

Fig. 5 shows how the pump response grows as a function of the magnitude of the $[K^+]_o$ change. TEP and intracellular apical membrane potentials were recorded in response to decreases in $[K^+]_o$ from 3.0 to 2.0 mM and to 1.0, 0.5, and 0.0 mM $[K^+]_o$ in the presence of Ba^{2+} . This range of $[K^+]_o$ changes was selected to mimic $[K^+]_o$ decreases evoked by light stimuli of increasing illuminance

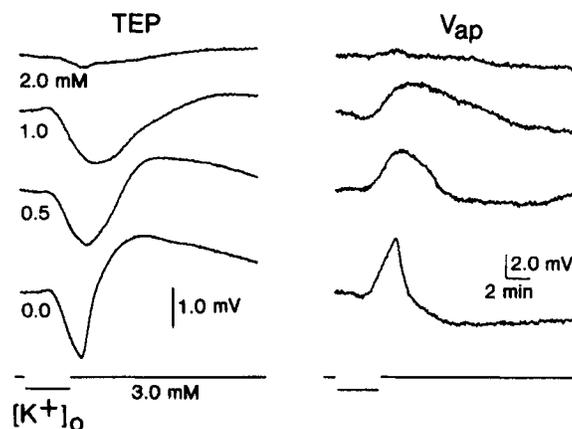


FIGURE 5. Responses to $[K^+]_o$ changes of increasing magnitude. TEP (left) and V_{ap} (right) responses evoked by $[K^+]_o$ changes in the presence of 1.0 mM Ba^{2+} . The timing of the $[K^+]_o$ changes, sequentially from 3.0 to 2.0, 1.0, 0.5, and 0.0 mM, appear below each TEP recording. Intracellular recordings were all from the same cell.

in the frog retina (Oakley and Green, 1976; Oakley, 1977; Fig. 11 below). When the $[K^+]_o$ change was made larger, the amplitude of the TEP decrease and the apical depolarization that generated it both increased.

For small decreases in $[K^+]_o$ (e.g., 3.0 to 2.0 mM), the apical membrane depolarized and TEP decreased, and these potentials recovered when $[K^+]_o$ was

¹ Poisoning the Na^+ - K^+ pump with ouabain decreased R_t and increased a (Griff, E. R., and Y. Shirao, unpublished; Miller and Steinberg, 1977b). The simplest explanation for the changes in R_t and a is that R_{ba} decreased. For a potential generated at the apical membrane, a decrease in only R_{ba} would make the apical and basal membrane responses smaller, and TEP responses larger. However, in most of the ouabain experiments, including many in which stable intracellular recording could not be done, the TEP response to a $[K^+]_o$ change was smaller in ouabain than in the control, although the apical response was larger. A decrease in the TEP response could be explained by a decrease in R_s , so that more of the apical response in ouabain was shunted to the basal membrane. Another explanation for the increase in a and decrease in R_t is that R_{ap} increased and R_s decreased; the combined effects of these resistance changes in ouabain would again make the apical response smaller. This suggests that the increase in the apical response seen in ouabain is a lower limit, and that the actual contribution of the pump under physiological conditions is larger.

returned back to 3.0 mM. For large changes (3.0 to 1.0 or 0.0 mM), the apical membrane hyperpolarized below baseline so that the TEP had an overshoot that appeared to result from the return of $[K^+]_o$ to 3.0 mM. Fig. 6, however, shows that the overshoot was actually a delayed response to the decrease in $[K^+]_o$ since it appeared when the tissue remained long enough in the low- $[K^+]_o$ test solution. In Fig. 6, $[K^+]_o$ was lowered from 3.0 to 0.0 mM for 17 min. At first, the TEP decreased, and the intracellular recordings show that a depolarization of the apical membrane generated this TEP decrease. Next, the TEP increased while the tissue was still in the low- $[K^+]_o$ solution; the intracellular recordings show that this TEP increase was generated by a hyperpolarization of the apical membrane. If ouabain is first added to the apical perfusate to poison the Na^+-K^+ pump (not shown), then both phases of this response are blocked, which suggests that both depend on the apical Na^+-K^+ pump. Details of the mechanism of this delayed response are now being investigated.

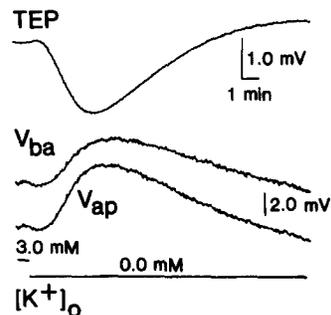


FIGURE 6. Responses to a sustained decrease in $[K^+]_o$. TEP, V_{ap} , and V_{ba} were recorded as in Fig. 2 in response to a decrease in $[K^+]_o$ from 3.0 to 0.0 mM in the continued presence of 1.0 mM Ba^{2+} . TEP first decreased because V_{ap} depolarized relative to V_{ba} . TEP then increased because V_{ap} hyperpolarized relative to V_{ba} .

Light Modulates the RPE Na^+-K^+ Pump in the Intact Retina

Light stimulation of the retina decreases $[K^+]_o$ in the subretinal space (Oakley and Green, 1976) and this normally hyperpolarizes the RPE apical membrane (Schmidt and Steinberg, 1971), increases TEP, and produces the RPE c-wave and retinal slow PIII (see below). To examine how this light-evoked $[K^+]_o$ decrease might modulate the RPE Na^+-K^+ pump, Ba^{2+} was added to the retinal perfusate of an *in vitro* preparation of neural retina-RPE-choroid to block apical g_{K^+} . Trans-tissue, TEP, and transretinal (V_R) potentials were monitored both in the dark and during responses to light stimuli (see Methods). The light-evoked trans-tissue response is equivalent to the corneal or vitreal ERG in the intact eye, and it is the sum of neural retina and RPE components. Of special interest for this study is the vitreal-positive ERG c-wave, which is the sum of a vitreal-negative component (slow PIII) that presumably originates from a hyperpolarization of the Müller cell and a larger, vitreal-positive RPE c-wave that originates from a hyperpolarization of the RPE apical membrane.

Fig. 7 shows trans-tissue ERGs evoked by a 30-s stimulus in control Ringer's

(left) and in a test solution containing 0.2 mM BaCl₂ (right). The control response consists of a positive b-wave followed by a positive c-wave; at stimulus offset, a positive d-wave is followed by a return to the dark-adapted DC potential (standing potential). When Ba²⁺ was added to the retinal perfusate, the standing potential in the dark decreased from a mean of 7.5 to 5.3 mV (six tissues). The time course of this decrease depended on the concentration of Ba²⁺, taking longer with lower concentrations. In 0.2 mM Ba²⁺, it took 40–60 min to reach its new level, which then remained stable for several hours. In Ba²⁺, light evoked a positive (but reduced) b-wave followed by a negative response that returned to the dark-adapted potential at stimulus offset. Upon return to control Ringer's, the standing potential and the normal light responses recovered. With 0.2 mM Ba²⁺, it took ~60 min for recovery (see Fig. 9, below). Thus, the major effect of Ba²⁺ was to decrease standing potential and invert the ERG c-wave, as first observed by Hu and Marmor (1984) in the intact eye of rabbit. Other g_K⁺ blockers, 4-aminopyridine (4-AP), tetraethylammonium (TEA), and Cs, did not

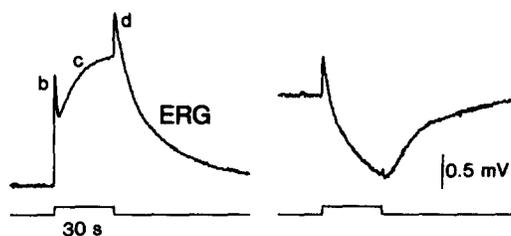


FIGURE 7. Effects of Ba²⁺ on the ERG. The ERG was recorded differentially across a preparation of neural retina-RPE-choroid. In control Ringer's (left), a 30-s stimulus, 4.1 ND (log attenuation), evoked a b-wave followed by a vitreal-positive c-wave; at stimulus offset, a d-wave was followed by a return to the dark-adapted baseline. The responses at the right were recorded ~60 min after BaCl₂ (0.2 mM) was added to the retinal perfusate. In Ba²⁺, a reduced b-wave was followed by a vitreal-negative potential, which returned to baseline at stimulus offset; the d-wave was reduced in Ba²⁺.

invert the c-wave, even with prolonged exposure to relatively high concentrations (4-AP, 6.0 mM; TEA, 4.0 mM; Cs, 10.0 mM).

To determine whether the effects of Ba²⁺ originated in the neural retina or in the RPE, a microelectrode was positioned in the subretinal space to measure TEP and V_R. When the retinal perfusate was switched to a test solution containing 0.2 mM Ba²⁺, the TEP decreased by 1.2 to 2.5 mV (five tissues measured ~60 min after introducing Ba²⁺), whereas V_R did not change. Thus, the decrease in standing potential produced by Ba²⁺ originated from the RPE as a decrease in TEP. Intracellular recordings showed that a depolarization of the RPE apical membrane generated this decrease in TEP. This effect of Ba²⁺ is similar to results presented above for the isolated RPE (Fig. 2B), and it provides evidence that Ba²⁺ blocks the RPE apical g_K⁺ when added to the retinal perfusate.

It was also anticipated that Ba²⁺ would block both the RPE c-wave and the Müller cell slow PIII and unmask a modulation of the RPE Na⁺-K⁺ pump. Fig. 8 shows ERG, TEP, and V_R responses evoked by 30-s stimuli presented at various

times after switching the retinal perfusate to one containing 0.2 mM Ba^{2+} . In each set of responses, the top trace, labeled 0, is the response in control perfusate and the bottom trace, labeled 60, is the steady state response in the presence of Ba^{2+} . As shown above in Fig. 7, the primary effect of Ba^{2+} on the ERG is to invert the c-wave. The TEP and V_R responses at 60 min show that this vitreal-negative ERG response originated solely from the RPE as a light-evoked decrease in TEP. The V_R response in Ba^{2+} was flat during this time.²

Fig. 8 also shows the transition of the responses as Ba^{2+} exerts its effects. The b-wave stabilized at a reduced amplitude (~50%) within 5 min, and the d-wave

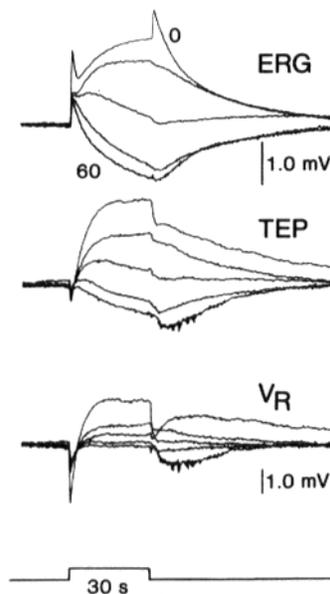


FIGURE 8. Effects of Ba^{2+} on the neural retinal and RPE components of the ERG. The ERG was recorded as in Fig. 7; the RPE component (TEP) and the neural retinal component (V_R) were measured simultaneously by recording differentially between a microelectrode positioned in the subretinal space and electrodes in the basal and retinal baths, respectively. Stimuli (60 s, 4.0 ND) were presented periodically after adding 0.2 mM Ba^{2+} to the retinal perfusate. The responses at time 0 were recorded in control perfusate; responses at time 60 min are representative of steady state responses in Ba^{2+} . The other responses were recorded 5, 15, and 30 min after adding Ba^{2+} . Same tissue as in Fig. 7.

was abolished at this time. (In the isolated retina, Ba^{2+} has no significant effect on the b-wave [Winkler and Gum, 1981; Oakley and Shimizaki, 1984].) The RPE c-wave and slow PIII, on the other hand, continued to change for ~60 min after Ba^{2+} . Slow PIII progressively decreased in amplitude and was abolished

² At stimulus offset, oscillations (2–4 Hz) were often recorded in both TEP and V_R . Since the amplitude of the V_R oscillation was slightly larger than TEP, we assume that the oscillations originated in the neural retina. In the isolated toad retina, Ba^{2+} , TEA, and 4-AP produce large depolarizing regenerative potentials that occur during the recovery phase of the rod light response (Fain et al., 1980). Oscillatory activity has also been described for horizontal cells (Normann and Pochobradsky, 1976).

after ~60 min. The RPE c-wave also decreased in amplitude, but, after ~15 min in Ba^{2+} , its time course became quite different: the TEP first increased but was then pulled down, and by 60 min a TEP decrease dominated the response. One can imagine that the positive TEP c-wave was abolished with approximately the same time course as the decrease in slow PIII, and that a TEP decrease was either unmasked or grew during this time. A blockade of both slow PIII and TEP c-wave responses by Ba^{2+} supports the hypothesis that both potentials are mediated by Ba^{2+} -sensitive K^+ conductances (Bolnick et al., 1979; Oakley et al., 1977).

To establish that the light-evoked TEP decrease in Ba^{2+} could represent a slowing of the RPE pump by a decrease in $[\text{K}^+]_o$, we first had to demonstrate that a light-evoked $[\text{K}^+]_o$ decrease persisted in our preparation in the presence

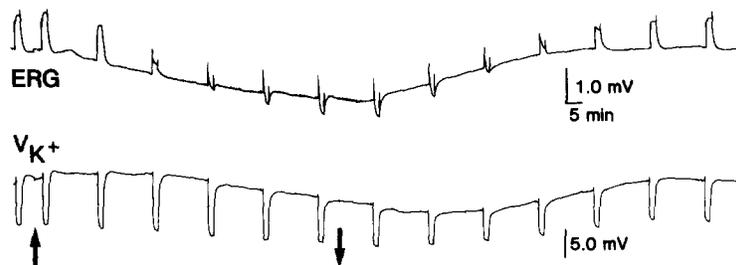


FIGURE 9. Effects of Ba^{2+} on subretinal $[\text{K}^+]_o$. The standing potential and ERGs were recorded as in Fig. 7. Subretinal $[\text{K}^+]_o$ was measured simultaneously with a double-barreled, K^+ -selective microelectrode positioned in the subretinal space; the signal recorded differentially between the K^+ barrel and the reference barrel, V_{K^+} , is proportional to $[\text{K}^+]_o$. The apical perfusate was switched to one containing 0.2 mM Ba^{2+} at the first arrow and switched back to control perfusate at the second arrow. 60-s stimuli (6.0 ND) were presented about every 10 min. The absolute $[\text{K}^+]_o$ levels were determined by rapidly withdrawing the K^+ microelectrode into the retinal perfusate (2.0 mM) and comparing the V_{K^+} change (7.8 mV) with the calibration curve for the K^+ microelectrode used in this experiment (not shown). This gave a value of 3.1 mM for $[\text{K}^+]_o$ in control perfusate and 2.4 mM at its minimum in Ba^{2+} .

of Ba^{2+} . Oakley (1983) previously showed that a light-evoked $[\text{K}^+]_o$ decrease persisted in the presence of Ba^{2+} in the isolated retina of the toad. Fig. 9 shows a continuous measurement of subretinal V_{K^+} and standing potential with light stimuli presented periodically. In control Ringer's, light produced its normal $[\text{K}^+]_o$ decrease, as indicated by the light-evoked decrease in V_{K^+} (Oakley and Green, 1976). When 0.2 mM BaCl_2 was added to the retinal perfusate (upward arrow), the dark-adapted steady state $[\text{K}^+]_o$ decreased from 3.1 to 2.4 mM; when Ba^{2+} was washed out (downward arrow), subretinal $[\text{K}^+]_o$ recovered. For three tissues in which absolute measurements were made, steady state $[\text{K}^+]_o$ in the dark was 3.0 ± 0.2 mM in control perfusate and 2.2 ± 0.2 mM in 0.2 mM Ba^{2+} . The changes in steady state $[\text{K}^+]_o$ did not parallel the changes in standing potential in Ba^{2+} . The standing potential and TEP (not shown) decreased before there was

any change in V_{K^+} and, after returning to normal Ringer's, both recovered, while V_{K^+} continued to decrease. Perhaps Ba^{2+} alters an apical K^+ conductance before it affects other retinal and/or RPE mechanisms that contribute to $[K^+]_o$.

In Fig. 9, responses were evoked periodically by light to see how Ba^{2+} altered the light-evoked V_{K^+} response. Two effects were evident. During the first 15 min in Ba^{2+} , the amplitude of the light-evoked V_{K^+} response increased, but with continued exposure to Ba^{2+} , it decreased and stabilized at a new reduced level after ~60 min. Thus, at the time when the inverted response was maximal and stable, a light-evoked decrease in subretinal $[K^+]_o$ persisted, although it was reduced in amplitude. The transient increase in the $[K^+]_o$ response could have been due to an increase in the photoreceptor's voltage response in Ba^{2+} (Brown and Flaming, 1978).

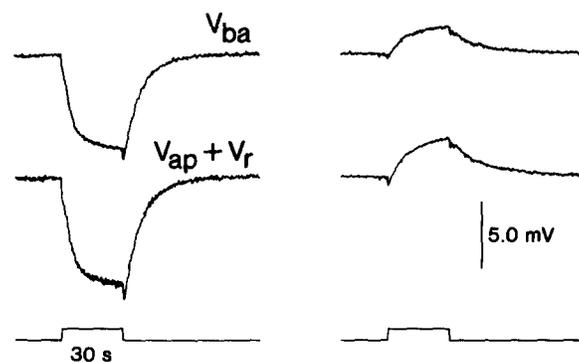


FIGURE 10. Effects of Ba^{2+} on light-evoked changes in RPE membrane potentials. The apical and basal membrane potentials ($V_{ap} + V_R$ and V_{ba} , respectively) were recorded simultaneously between an intracellular microelectrode and electrodes in the retinal and basal baths in response to a 30-s stimulus (5.0 ND). The apical membrane potential was contaminated by extracellular voltages generated in the neural retina (see text). In control perfusate (left), light evoked a large apical hyperpolarization and smaller basal hyperpolarization. The responses on the right were recorded from the same cell ~60 min after switching the retinal perfusate to one containing 0.2 mM Ba^{2+} . In Ba^{2+} , light evoked a depolarization of the apical membrane and a smaller depolarization of the basal membrane.

If the light-evoked decrease in TEP represents a slowing of the RPE apical Na^+K^+ pump as it does in the isolated RPE, then it should be generated by a depolarization of the RPE apical membrane. Fig. 10 compares intracellular RPE recordings in the presence and absence of Ba^{2+} . In the control solution, light evoked a large hyperpolarization of the apical membrane and a smaller hyperpolarization of the basal membrane, as previously described (Oakley et al., 1977; Oakley, 1977). The retinal perfusate was then switched to one containing 0.2 mM Ba^{2+} . After the membrane potentials and TEP had reached stable levels in Ba^{2+} , the light stimulus was repeated. In the presence of Ba^{2+} , light evoked a depolarization of the apical membrane (Fig. 10, $V_{ap} + V_R$) and a smaller depolarization of the basal membrane. Since no voltage is generated across the neural retina during this time period (see Fig. 8, V_R), the depolarization must originate

at the apical membrane. These are the membrane responses expected for a slowing of the apical $\text{Na}^+\text{-K}^+$ pump (see Methods, Eqs. 3 and 4). The largest pump response recorded was a 6.0-mV light-evoked apical depolarization, which was near saturation for this effect (3.0 neutral density [ND]).

To estimate the expected increase in R_{ap} caused by Ba^{2+} , pulses of current were injected across the tissue and the appropriate voltage drops were measured (see Methods). Table II shows values of a and R_{i} in the presence and absence of Ba^{2+} for two dark-adapted retina-RPE-choroid preparations. Ba^{2+} caused an increase in R_{i} and an increase in a . Such results are most simply explained by an increase in apical resistance. Ba^{2+} also increased the resistance of the neural retina (R_{R}), but this could account for only 15% of the increase in a and R_{i} . As in the isolated RPE (see Appendix), prolonged exposure to Ba^{2+} caused a subsequent decrease in R_{i} , accompanied by a continued increase in a . Thus, in addition to the increase in apical resistance, there was a subsequent decrease in either basal or paracellular resistances.

TABLE II
Effects of Ba^{2+} on RPE Resistance

Tissue	Control	<4 min in Ba^{2+}	>20 min in Ba^{2+}
G333.1			
a	0.27	0.29	0.41
R_{i} (k Ω)	3.7	3.8	3.5
G333.2			
a	0.17	0.21	0.41
R_{i} (k Ω)	3.8	3.9	3.5

Retina-RPE-choroid preparation. Current pulses were injected across the tissue at various times after switching the retinal perfusate to one containing 0.2 mM Ba^{2+} . The total RPE resistance, R_{i} , and the ratio $a = (R_{\text{ap}} + R_{\text{R}})/R_{\text{ba}}$ were calculated from the appropriate iR drops. When measured within 4 min of introducing Ba^{2+} , both a and R_{i} increased; in the steady state condition, measured >20 min after introducing Ba^{2+} , a increased but R_{i} decreased.

Properties of the Light-evoked Pump Response

The light-evoked decrease in the electrogenic pump is further characterized in Fig. 11, where responses to increasing stimulus illuminance are compared in control (A) and Ba^{2+} -containing (B) retinal perfusate. In the control, the dark-adapted c-wave and the subretinal V_{K^+} decrease grew over at least 5.5 log units; a response was easily detectable with 7.5 log units attenuation and the amplitude of both the V_{K^+} and the ERG responses saturated at ~ 2.0 log units attenuation. Responses in Ba^{2+} also grew with increasing illuminances but over a smaller range. A response was easily detectable at 7.5 log units attenuation, but saturated at 4 log units attenuation.

As indicated by the V_{K^+} responses in Fig. 9, the dark-adapted subretinal $[\text{K}^+]_{\text{o}}$ was lower in Ba^{2+} than in control, and for any particular stimulus illuminance, the light-evoked decrease was always smaller. This reduction of the V_{K^+} response, however, cannot account for the unmasking of an inverted response in Ba^{2+} since there was no level of illumination in Ba^{2+} at which a vitreal-positive c-wave

occurred. Stimuli that produced approximately equal $[K^+]_o$ decreases, such as 5.0 in control and 3.0 ND (log attenuation) in Ba^{2+} did not produce similar responses; a positive c-wave was observed in control and an inverted response in Ba^{2+} .

Fig. 12 shows ERG and V_{K^+} responses to stimuli of increasing duration in control (A) and Ba^{2+} -containing (B) perfusate. As previously shown by Oakley and Steinberg (1982), in response to stimuli longer than 30 s, the c-wave reaches a peak and then starts to decay while the light is on (Fig. 12A, control). The decay of the c-wave reflects a reaccumulation of $[K^+]_o$ during long stimuli. Thus,

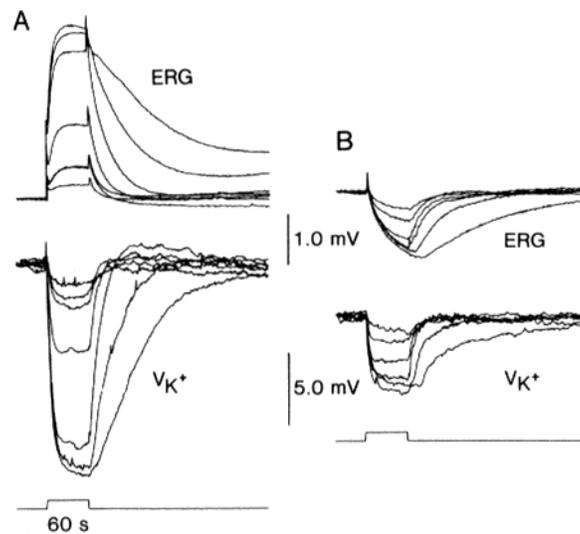


FIGURE 11. Intensity series. ERG and V_{K^+} were recorded as in Fig. 9 in response to 60-s stimuli of increasing illuminance. Responses in control perfusate (A) were evoked by 60-s stimuli attenuated by 7.5, 7.0, 6.0, 5.0, 4.0, 3.0, and 2.0 ND. The responses in B were recorded starting ~180 min after switching the retinal perfusate to one containing 0.2 mM Ba^{2+} in response to stimuli attenuated by 7.5, 7.0, 6.0, 5.0, 4.0, and 3.0 ND. Assuming that the dark-adapted $[K^+]_o$ was 3.0 mM in control perfusate, the maximum V_{K^+} response corresponds to a decrease to 1.2 mM. In Ba^{2+} , steady state $[K^+]_o$ decreased to 1.8 mM and the maximum V_{K^+} response corresponds to a decrease to 1.3 mM.

the c-wave increases as $[K^+]_o$ falls and decays as $[K^+]_o$ reaccumulates. In Ba^{2+} (Fig. 12B), the pump response (vitreal-negative potential) grew in amplitude with stimulus duration up to 120 s. In this tissue, the response did not recover toward the dark-adapted level while the light was on. In about half of the tissues, however, such a recovery was evident (Fig. 12C); the vitreal-negative response reached a minimum at ~60 s and then recovered toward the baseline. Note that this occurred in Ba^{2+} even though the V_{K^+} response showed no reaccumulation (Oakley, 1983). The mechanism of this recovery toward baseline is currently under investigation.

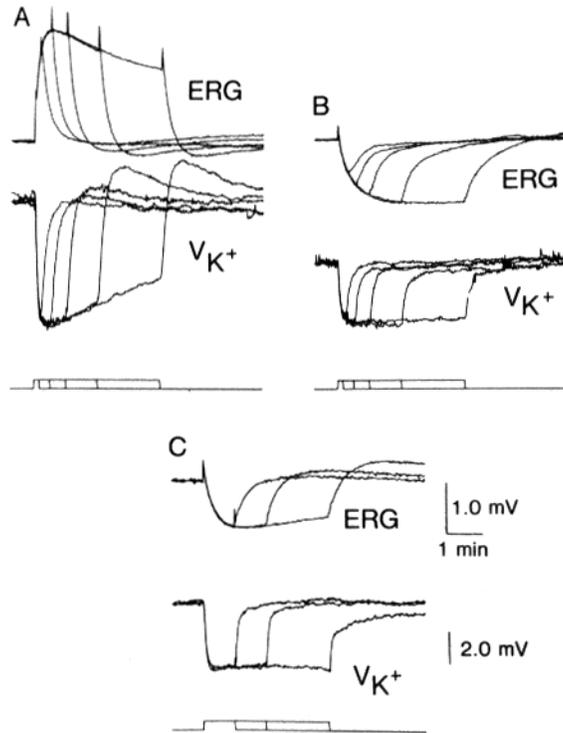


FIGURE 12. Duration series. ERG and V_{K^+} were recorded as in Fig. 9 in response to stimuli of increasing duration. The responses in *A* were recorded in control perfusate in response to stimuli of 10, 30, 60, 120, and 240 s, all 5.0 ND. The responses in *B* were evoked by identical stimuli, in the same tissue, starting ~240 min after switching the retinal perfusate to 0.2 mM Ba^{2+} . The same tissue and K^+ microelectrode penetration as in Fig. 11 were used. The responses in *C* were also recorded in 0.2 mM Ba^{2+} , but from a different tissue, in response to stimuli of 60, 120, and 240 s, 5.0 ND.

DISCUSSION

This paper presents electrophysiological evidence in support of the hypothesis that a decrease in K^+ concentration in the subretinal space slows the Na^+-K^+ pump located on the RPE apical membrane. Since the pump normally hyperpolarizes the apical membrane, a slowing of the pump depolarizes the apical membrane and decreases TEP. This apical depolarization is not observed under control conditions, because a decrease in $[K^+]_o$ also hyperpolarizes the apical membrane as the K^+ equilibrium potential becomes more negative, and this apical hyperpolarization normally masks the pump response. The contribution of the Na^+-K^+ pump was demonstrated experimentally in two ways using the isolated RPE-choroid preparation of the bullfrog. First, if the K^+ conductance of the apical membrane was blocked with Ba^{2+} , the normal apical hyperpolarization was also blocked and a decrease in $[K^+]_o$ evoked an apical depolarization that was blocked by ouabain or strophanthidin. Second, if the tissue was first

treated with ouabain to inhibit the $\text{Na}^+\text{-K}^+$ pump, a decrease in $[\text{K}^+]_o$ (Ba^{2+} absent) evoked a larger apical hyperpolarization than the response without ouabain, which suggests that an apical depolarization that occurs normally had been blocked.

Previous studies on the isolated RPE also indicated that small changes in $[\text{K}^+]_o$ modulate the RPE apical $\text{Na}^+\text{-K}^+$ pump. Miller and Steinberg (1982) showed that the net rate of apical-to-basal K^+ transport, which was inhibited by ouabain, increased monotonically as $[\text{K}^+]_o$ was increased from 0.2 to 5.0 mM. Taurine transport, which also depends on the $\text{Na}^+\text{-K}^+$ pump to maintain a Na^+ gradient, also increased monotonically with increasing $[\text{K}^+]_o$ (Miller and Steinberg, 1979; Ostwald and Steinberg, 1981). The electrophysiological evidence presented in this paper demonstrated a modulation of the pump by 0.0–3.0 mM $[\text{K}^+]_o$. Additional experiments (Griff, E. R., and Y. Shirao, unpublished) indicate that pump modulation by $[\text{K}^+]_o$ saturates at ~ 5.0 mM, in agreement with the above transport studies.

In the retina-RPE-choroid preparation, as in vivo, light evokes a decrease in subretinal $[\text{K}^+]_o$, which normally hyperpolarizes the RPE apical membrane and generates an increase in TEP (TEP *c*-wave). Oakley (1977) showed that these RPE responses resulted solely from the decrease in $[\text{K}^+]_o$, and suggested that changes in the concentrations of other ions do not make a significant contribution. When Ba^{2+} was added to the retinal perfusate, the light-evoked $[\text{K}^+]_o$ decrease survived, but in this case the apical membrane depolarized and TEP decreased. Since Ba^{2+} did not unmask any other apical conductances in the isolated RPE-choroid preparation and because the light-evoked responses in the presence of Ba^{2+} are qualitatively the same as those in the isolated RPE-choroid, we conclude that they too represent a slowing of the RPE $\text{Na}^+\text{-K}^+$ pump. Since we could also demonstrate in the isolated RPE-choroid that the pump was normally modulated by $[\text{K}^+]_o$ (i.e., without using Ba^{2+}), we further propose that the normal TEP *c*-wave represents at least two responses of the RPE to the light-evoked decrease in $[\text{K}^+]_o$: an apical hyperpolarization as E_K becomes more negative and an apical depolarization because the pump is slowed. From these experiments, we could not estimate the size of the pump contribution to the *c*-wave. It could be quite small. However, as suggested below, our experiments may actually underestimate the normal contribution of the pump.

Although both Ba^{2+} and ouabain unmasked a response that reflected a modulation of the pump by $[\text{K}^+]_o$ in the isolated RPE, the amplitude of the response in the presence of these agents was not necessarily the same as would occur normally (i.e., without Ba^{2+} or ouabain). As shown by Eqs. 3 and 4, the amplitude of the pump response recorded across either of the RPE membranes or across the RPE itself depends on both the pump current and the RPE resistances, and both Ba^{2+} and ouabain alter these resistances. There are several indications that the amplitudes of responses in the presence of Ba^{2+} and ouabain actually underestimate the pump's response under physiological conditions. If the addition of Ba^{2+} had simply increased R_{ap} as it blocked the apical K^+ conductance, then the responses recorded in Ba^{2+} would overestimate the decrease in pump current. For example, in a study of photoreceptors (Torre, 1982), Cs^+ was added to block conductances and this artificially increased the voltage produced by photorecep-

tor pump current. In our study, Ba^{2+} caused an apparent increase in R_{ap} and a decrease in R_{ba} , and in the steady state, the decrease in R_{ba} dominated so that R_t decreased. In this case, the response seen in the presence of Ba^{2+} (maximum, 3.7 mV) underestimates the modulation of the pump current. It has also been suggested that Ba^{2+} reduces the Na^+ -to- K^+ coupling ratio, and therefore the electrogenicity, of the Na^+ - K^+ pump (Walter and Sillman, 1984). Ouabain also produced changes in RPE resistance, decreasing R_s and/or R_{ba} so that the maximum amplitude of the pump response estimated by comparing the response evoked by a decrease in $[K^+]_o$ from 3.0 to 1.0 mM in the presence and absence of ouabain, 2.2 mV, probably also underestimates the pump response that occurs under physiological conditions. With the retina-RPE-choroid preparation, the maximum light-evoked response in Ba^{2+} for which we had an intracellular recording was a 6.0-mV apical depolarization. Since R_t decreased in Ba^{2+} (see Table II), we assume that a decrease in R_{ba} dominated, so that the response in Ba^{2+} underestimates the pump response that normally occurs (i.e., without Ba^{2+}). The smaller pump response in the isolated RPE preparation may have resulted from damage to the apical membrane during dissection.

A pump response of the magnitude indicated above will not greatly change the description of the passive ionic properties of the apical membrane, such as the relative g_{K^+} , because these calculations are based primarily on $[K^+]_o$ changes above 5.0 mM, where the pump is not modulated (Miller and Steinberg, 1977a, 1979). The pump response could make a significant contribution to c-wave amplitudes, however, since a small change in the apical response can produce a large percent change in the ERG, particularly if it is not accompanied by a change in V_R (Linsenmeier and Steinberg, 1983). For example, a 2–3-mV apical depolarization that could reduce the RPE c-wave by 1.0 mV (an ~20% change) would also reduce the ERG c-wave by 1.0 mV (a 100% change).

APPENDIX

RPE Resistance Changes in Ba^{2+}

To estimate the expected increase in R_{ap} produced by Ba^{2+} , pulses of current were injected across the tissue and the appropriate voltage drops (iR) were monitored (see Methods). The iR drop across the tissue is proportional to the total resistance, R_t , and the ratio of the iR drops across the apical and basal membranes to the ratio of their resistances, $R_{ap}/R_{ba} = a$. When 0.2 mM Ba^{2+} (Fig. 13, open circles) was added to the apical perfusate (arrow), a increased and R_t remained unchanged or increased slightly; with continued exposure to Ba^{2+} , a continued to increase and R_t always decreased.

An increase in a could result from an increase in R_{ap} , a decrease in R_{ba} , or both. A decrease in R_t indicates that at least one of the RPE resistances must have decreased. The simplest explanation for the increase in a and the decrease in R_t is a decrease in R_{ba} . Since we expected R_{ap} to increase in Ba^{2+} , we suspected that Ba^{2+} altered at least two RPE resistances—for example, increasing R_{ap} and decreasing R_{ba} . This hypothesis gets some support from the resistance measurements during the first few minutes after introducing Ba^{2+} , in which a increased but R_t did not change. Since R_t did not change, one RPE resistance must have increased and another decreased in Ba^{2+} .

After the first few minutes in Ba^{2+} , R_t decreased, although a continued to increase. Thus, during this period, a decrease in R_{ba} or R_s dominated, so that R_t decreased. When

TEP, R_t , and a stabilized at new steady state values in Ba^{2+} , a had increased from a mean of 0.2 ± 0.1 (SD) to 0.6 ± 0.2 (SD; $n = 7$), while R_t decreased from 4.0 ± 0.9 (SD) to 3.6 ± 0.8 k Ω (SD) (see Table III).

Measurements of the sequence of resistance changes were more clearly observed using higher concentrations of Ba^{2+} , and these results support the hypothesis that an increase in R_{ap} occurs and is followed or accompanied by a decrease in R_{ba} and/or R_t . The closed circles in Fig. 13 show the results of switching to a test solution containing 2.0 mM Ba^{2+} .

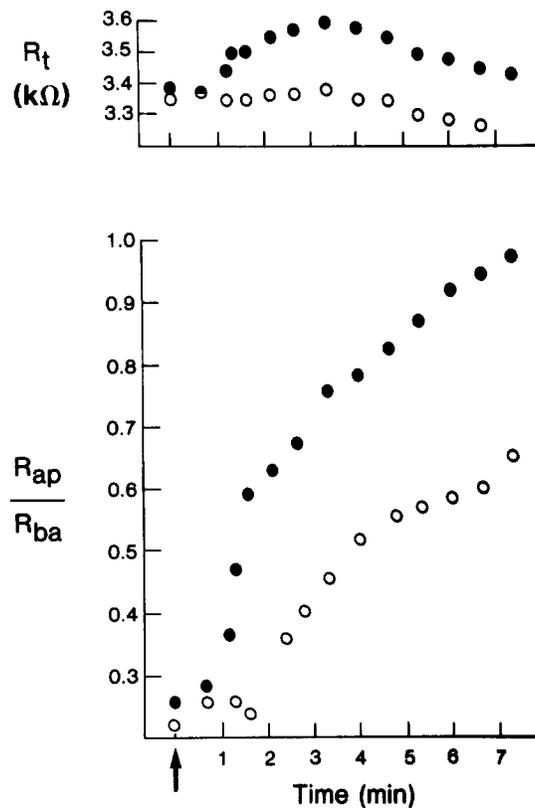


FIGURE 13. The effects of Ba^{2+} on RPE resistances. R_t and the ratio $a = R_{ap}/R_{ba}$ are plotted as a function of time after switching the apical perfusate to one containing Ba^{2+} (arrow). The open circles show the sequence of resistance changes with 0.2 mM Ba^{2+} and the filled circles show the sequence of changes with 2.0 mM Ba^{2+} . See text for details. Intracellular measurements used to calculate R_{ap}/R_{ba} were obtained from the same RPE cell for all points in both traces.

Initially, R_t and a both increased, which is consistent with an increase in R_{ap} . After several minutes in Ba^{2+} , R_t started to decrease, while a continued to increase, which suggests that a subsequent decrease in R_{ba} now dominates.

We obtained further evidence that a decrease in R_{ba} produced the delayed decrease in R_t by blocking the basal membrane conductance with Ba^{2+} . 5 mM Ba^{2+} was necessary to block basal g_{K^+} . The evidence that Ba^{2+} blocked basal g_{K^+} is that it depolarized the basal membrane, increased R_t , decreased a , and completely blocked the response of the basal

TABLE III
Effects of Ba^{2+} on RPE Resistances

Tissue	Control		0.2 mM Ba^{2+}	
	a	R_t	a	R_t
		$k\Omega$		$k\Omega$
G317	0.14	4.0	0.58	3.8
G318	0.48	2.6	0.57	3.8
G321.1	0.23	3.6	1.0	3.4
G321.2	0.12	3.4	0.28	3.4
G327	0.15	4.8	0.54	4.3
G328	0.13	4.2	0.4	3.8
G329	0.15	4.0	0.55	3.6
Mean	0.20	4.0	0.55	3.6
SD	0.13	0.9	0.23	0.8

Isolated RPE-choroid preparation. Current pulses were injected across the tissue; R_t and a were calculated from the appropriate iR drops. Measurements were made before and ~60 min after switching the apical perfusate to one containing 0.2 mM Ba^{2+} .

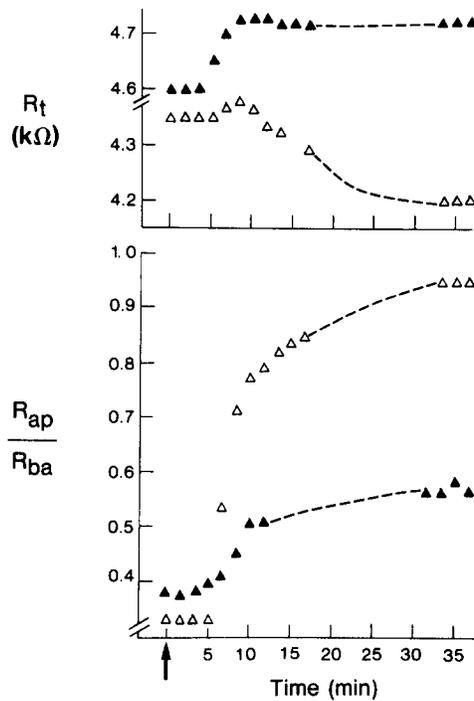


FIGURE 14. The effects of basal Ba^{2+} on the RPE resistance. R_t and the ratio $a = R_{ap}/R_{ba}$ are plotted as a function of time after switching the apical perfusate to one containing 0.2 mM Ba^{2+} (arrow). The open triangles show measurements with control basal perfusate. The solid triangles show measurements made starting ~30 min after switching the basal perfusate to one containing 5.0 mM Ba^{2+} . Intracellular measurements used to calculate R_{ap}/R_{ba} were obtained from the same RPE cell for all points in both traces.

membrane to an increase in $[K^+]_o$ in the basal perfusate from 2.0 to 10.0 mM. Fig. 14 compares the change in a and R_i evoked by 0.2 mM apical Ba^{2+} in the presence and absence of 5.0 mM basal Ba^{2+} . The response in control basal perfusate is shown by the open triangles. In the presence of basal Ba^{2+} (closed triangles), apical Ba^{2+} produced primarily an increase in both a and R_i ; the delayed decrease in R_i normally observed after 3–4 min in apical Ba^{2+} was almost completely blocked. The amplitude of the increase in a produced by apical Ba^{2+} was reduced since now only an increase in R_{ap} , rather than both an increase in R_{ap} and a decrease in R_{ba} , contributed. This smaller increase in a is a lower bound on the actual increase in R_{ap} produced in 0.2 mM Ba^{2+} since preliminary evidence suggests that 5.0 mM Ba^{2+} in the basal perfusate also blocks at least some of the apical K^+ conductance.³ Thus, the actual increase in R_{ap} evoked by apical Ba^{2+} is between the increase in a observed with and without basal Ba^{2+} .

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³ In addition to the effects of basal Ba^{2+} on the basal membrane potential, R_i , a , and the response to changes in basal $[K^+]_o$ described above, there was also a delayed effect of basal Ba^{2+} on the apical membrane. Basal Ba^{2+} produced a delayed depolarization of the apical membrane, an increase in R_i , an increase in a , and a decrease in the apical response to changes in apical $[K^+]_o$. These delayed effects are all consistent with a decrease in apical g_{K^+} . Preliminary experiments in the retina-RPE-choroid preparation provide additional evidence that basal Ba^{2+} decreases apical g_{K^+} . When the basal perfusate was switched to one containing 5.0 mM Ba^{2+} , the ERG c-wave inverted, a response identical to the one obtained when Ba^{2+} was added to the retinal perfusate.

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