Changes in Apical $[K^+]$ Produce Delayed Basal Membrane Responses of the Retinal Pigment Epithelium in the Gecko

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ABSTRACT We describe here a new retinal pigment epithelium (RPE) response, a delayed hyperpolarization of the RPE basal membrane, which is initiated by the light-evoked decrease of $[K^+]_o$ in the subretinal space. This occurs in addition to an apical hyperpolarization previously described in cat (Steinberg et al., 1970; Schmidt and Steinberg, 1971) and in bullfrog (Oakley et al., 1977; Oakley, 1977). Intracellular and extracellular potentials and measurements of subretinal $[K^+]_o$ were recorded from an in vitro preparation of neural retina-RPE-choroid from the lizard *Gekko gekko* in response to light. Extracellularly, the potential across the RPE, the transepithelial potential (TEP), first increased and then decreased during illumination. Whereas the light-evoked decrease in $[K^+]_o$ predicted the increase in TEP, the subsequent decrease in TEP was greater than predicted by the reaccumulation of $[K^+]_o$. Intracellular RPE recordings showed that a delayed hyperpolarization generated at the RPE basal membrane produced the extra TEP decrease. At light offset, the opposite sequence of membrane potential changes occurred. RPE responses to changes in $[K^+]_o$ were studied directly in the isolated gecko RPE-choroid. Decreasing $[K^+]_o$ in the apical bathing solution produced first a hyperpolarization of the apical membrane, followed by a delayed hyperpolarization of the basal membrane, a sequence of membrane potential changes identical to those evoked by light. Increasing $[K^+]_o$ produced the opposite sequence of membrane potential changes. In both preparations, the delayed basal membrane potentials were accompanied by changes in basal membrane conductance. The mechanism by which a change in extracellular $[K^+]$ outside the apical membrane leads to a polarization of the basal membrane remains to be determined.

INTRODUCTION

The retinal pigment epithelium (RPE) has two separate membranes: an apical membrane that faces the photoreceptors of the neural retina and a basal membrane that faces the choroid. Previous work on in vitro preparations of frog RPE showed that the apical membrane has an appreciable K^+ conductance (Miller and Steinberg, 1977), so that its potential is a function of the extracellular potassium concentration at the apical membrane, apical $[K^+]_o$. Since apical $[K^+]_o$ decreases in response to absorption of light by photoreceptors (Oakley and Green, 1976),

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the apical membrane hyperpolarizes (cat, Steinberg et al., 1970; Schmidt and Steinberg, 1971; bullfrog, Oakley et al., 1977; Oakley, 1977). If illumination is maintained, the initial decrease in apical $[K^+]_o$ reaches a minimum and then rises to a new plateau level as K^+ reaccumulates (cat, Steinberg et al., 1980; bullfrog, Oakley and Steinberg, 1982). In frog, the apical membrane potential follows these changes, and across the epithelial layer this is observed as an increase in the transepithelial potential (TEP) to a peak and then a decrease to a plateau (Oakley and Steinberg, 1982). These changes in TEP form the RPE component of the c-wave of the DC electroretinogram (DC ERG) that is recorded across the tissue (neural retina-RPE-choroid); in frog they result only from changes in the potassium equilibrium potential across the apical membrane. Apart from this apical response to K^+, no other direct responses to retinal illumination have been defined in the frog RPE.

The DC ERGs of the vertebrate classes Reptilia, Aves, and Mammalia are made more complex by a series of light-dependent potentials that follow the c-wave (Kikawada, 1968). There is first a decrease in potential, the fast oscillation (Kolder and Brecher, 1966; Täumer et al., 1976), and then a large slow increase in potential, the light peak (Marmor and Lurie, 1979). Recently (Griff and Steinberg, 1982*a*), we have demonstrated that the light peak is generated at the RPE basal membrane by a depolarization that is associated with an apparent increase in basal membrane conductance; this basal depolarization has been demonstrated in both the reptile *Gekko gekko* and in the cat (Linsenmeier and Steinberg, 1982; Steinberg et al., 1983). Thus, whereas the light-evoked potentials recorded across the frog RPE are generated solely by the apical membrane, in gecko and cat both apical and basal membranes generate responses.

Because of the complexity of the RPE responses described above, it was of interest to study the K^+ response of the RPE of gecko. First, studying the RPE in isolation from the neural retina, we changed apical $[K^+]_o$; in addition to the response expected from the change in the equilibrium potential at the apical membrane, we observed a new response of the basal membrane. Next, by studying the RPE with the neural retinal attached, we found that a similar basal membrane response followed the light-evoked decrease in apical $[K^+]_o$. The previously undescribed response is a delayed hyperpolarization of the basal membrane. Thus, in gecko, changes in apical $[K^+]_o$ are communicated to the RPE basal membrane. The following paper (Linsenmeier and Steinberg, 1984) demonstrates a similar response in cat, and we have also observed it in the chicken (E. R. Griff, unpublished observations). Thus, a K^+-dependent, delayed basal response is a property common to reptiles, birds, and mammals.

METHODS

Preparation

Tokay geckos, *Gekko gekko*, were obtained from Charles Sullivan, Inc., Nashville, TN, and kept according to the suggestions of Pawley (1966). Before an experiment, animals were refrigerated (10°C) to facilitate handling and anesthetized by packing in ice; after enucleation, the animals were killed. Two preparations were used. To dissect a tissue

consisting of neural retina-RPE-choroid, the animal was first light-adapted to minimize retinal detachment. The eye was enucleated under red light and submerged in perfusate, and a section of sclera was removed. A 5-mm^2 piece of tissue was dissected free from the vitreous and mounted in a chamber as previously described for bullfrog (Miller and Steinberg, 1977; Oakley, 1977). To isolate an RPE-choroid preparation, the animal was first dark-adapted to facilitate removal of the retina; the eye was enucleated and sectioned behind the lens, and the posterior portion was submerged in perfusate. The retina was removed and discarded and the remaining RPE-choroid was dissected free from the sclera and mounted in the chamber.

In both preparations, the two sides of the tissue, retinal (or apical) and choroidal, were continuously superfused at 2–5 ml/min by a gravity feed system from two large reservoirs. The area of tissue exposed to the perfusate was 0.07 cm^2. The perfusate was a modified Ringer solution having the following composition (mM): 82.5 NaCl, 27.5 $NaHCO_3$, 2.0 KCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 25.0 glucose, bubbled with 95% O_2 and 5% CO_2, pH 7.4. The solution could be switched to one with altered apical $[K^+]_o$ (1.0, 3.0, or 5.0 mM KCl) by means of a three-way valve.

Electrodes and Recording

The trans-tissue potential (transepithelial potential for the isolated RPE-choroid preparation) was measured differentially between two calomel electrodes that were connected to the retinal (apical) and choroidal baths, respectively, by a pair of agar-Ringer bridges. Current pulses (1–5 μA for 1.0 s) were injected across the tissue through two silver chlorided wires connected to each bath by a second pair of bridges.

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 100 MΩ. Unity-gain preamplifiers (model 1090; Winston Electronics, San Francisco, CA) with input impedances of 10^{14} Ω were used to measure the potentials between the microelectrode and the retinal and/or choroidal calomels (see Griff and Steinberg, 1982*a*, Fig. 1). When the microelectrode was positioned in the subretinal space, we recorded the transretinal potential between the microelectrode and the retinal calomel, and the TEP between the microelectrode and the choroidal calomel. A second microelectrode was placed intracellularly to record the apical and basal membrane potentials, V_{ap} and V_{ba}, respectively. We recorded V_{ba} between the intracellular microelectrode and the choroidal calomel; we obtained V_{ap} by subtracting V_{ba} from TEP (see Griff and Steinberg, 1982*a*, Fig. 2).

K^+-selective microelectrodes were constructed from double-barreled glass tubing (fused side by side, each barrel 1.0 mm OD), as described by Oakley (1977). One barrel was filled with a K^+ exchanger solution (477317; Corning Glass Works, Science Products Div., Corning, N Y) and the second (reference) barrel with 5 M LiCl. K^+ electrodes were beveled until the reference barrel had a resistance of 50–100 MΩ; tip size was ~1 μm. This electrode was positioned in the subretinal space, just outside the apical membrane, to measure apical $[K^+]_o$. The potential, V_{K^+}, between the K^+ barrel and the reference barrel was proportional to the $[K^+]_o$ (Oakley, 1977). The potential between the reference barrel and the choroidal calomel gave a simultaneous measure of the TEP. Each K^+-selective electrode was calibrated before an experiment in solutions of varying $[K^+]_o$ but constant $[K^+]_o + [Na^+]_o = 112$ mM. Increasing $[K^+]_o$ from 2 to 5 mM typically produced a V_{K^+} of 10–15 mV.

White light from a 100-W tungsten halogen lamp (3,300°K) was used to stimulate the retina-RPE-choroid preparation. The lamp filament was focused by aspheric condensing lenses, passed through a water heat filter, and directed at the preparation by a front-surface mirror. Unattenuated, the stimulus delivered 92 mW/cm^2 to the preparation.

RESULTS

The results are divided into two sections: the first describes responses evoked by direct manipulation of apical $[K^+]_o$ in the isolated RPE-choroid; the second describes light-evoked responses from a preparation of neural retina-RPE-choroid. In both preparations we recorded changes in apical and basal membrane potentials and the resulting change in TEP. A brief discussion of the equivalent electrical circuit of the RPE is presented first.

Theory

To determine how changes in TEP can arise, it is important to understand what conclusions can be drawn from intracellular recordings. This discussion follows the electrical analysis of the RPE performed by Miller and Steinberg (1977) and

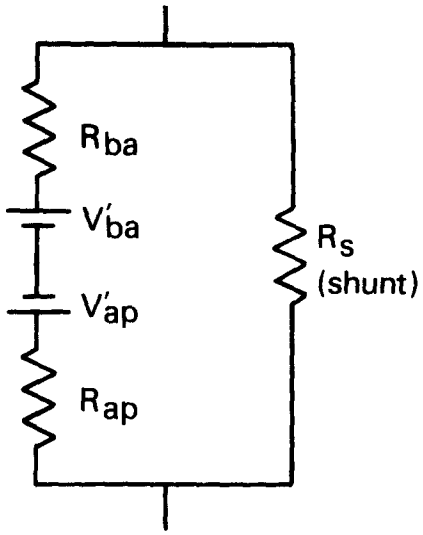

FIGURE 1. Equivalent circuit of the RPE. The resistors represent the apical membrane, which faces the retina, the basal membrane, which faces the choroid, and the paracellular shunt. Batteries V'_{ap} and V'_{ba} represent voltages generated at the apical and basal membranes. The apical and basal membrane potentials are given by: $V_{ap} = V'_{ap} - i_s R_{ap}$ and $V_{ba} = V'_{ba} + i_s R_{ba}$, where i_s is the shunt current (see text). In the dark $V_{ap} > V_{ba}$, so that the subretinal space is positive relative to the choroid and $+i_s$ flows outward across R_{ap}, through R_s, and inward across R_{ba}.

Oakley (1977), and discussed further by Griff and Steinberg (1982*a*) and Linsenmeier and Steinberg (1983). Relevant equations will be found in those papers.

The RPE apical and basal membranes are electrically separated by the finite resistance of the paracellular shunt, R_s, as shown in Fig. 1. Since the apical membrane potential, V_{ap} (mean = −85 ± 7 mV [SD], n = 18 cells), is more hyperpolarized than the basal potential, V_{ba} (mean = −69 ± 8 mV [SD], n = 18 cells), a "shunt" current flows around the RPE circuit. This current will change whenever a change in potential is initiated at one of the cell membranes. At the

other cell membrane, the change in current produces a passive change in membrane potential that is of the same sign but smaller in amplitude. For example, an apical $[K^+]_o$ decrease hyperpolarizes the apical membrane and, because of the change in shunt current, produces a smaller passive hyperpolarization of the basal membrane.

The TEP is the difference between apical and basal membrane potentials (mean TEP = 19 ± 6 mV, n = 66). Changes in TEP can be initiated at either cell membrane, and if no shunting occurred, the change in TEP would equal the change in apical or basal membrane potential. Because shunting does exist, both membranes polarize in the same direction, and the change in TEP is reduced since it is the difference between the new potentials at both cell membranes. In the simplest case, a potential is initiated at only one membrane and passively shunted to the other. If both membranes initiate potential changes at the same time, the potential measured at each membrane is the sum of the potential generated at that membrane and the potential shunted from the other membrane. For example, in the results presented below (Fig. 9, period 2), a hyperpolarization is generated at the basal membrane, whereas a depolarization is generated at the apical membrane. In this example, the hyperpolarization that is shunted to the apical membrane is larger than the depolarization generated there, so that we measure a net apical hyperpolarization. Thus, the basal membrane hyperpolarization dominates during this period and the TEP decreases. If the apical membrane hyperpolarizes relative to the basal or if the basal membrane depolarizes relative to the apical, TEP will increase. If the basal membrane hyperpolarizes relative to the apical or if apical membrane depolarizes relative to the basal, TEP will decrease.

A change in TEP can also be produced if a potential is generated across the paracellular shunt. This could be produced by a change in the concentration of an ion to which this pathway is selectively permeable. Such a potential would also change the amount of current flowing in the RPE circuit and therefore alter the potential at each membrane. For example, if a shunt potential increased the TEP, more current would flow in across the apical membrane, hyperpolarizing it, and out across the basal membrane, depolarizing it. Thus, a potential generated across the paracellular shunt would polarize the RPE membranes in opposite directions.

Responses of the Isolated RPE-Choroid

In this section we describe the responses to increases and decreases in apical $[K^+]_o$. The "normal" $[K^+]_o$ was defined as 2 mM in both the apical and basal bathing solutions (Miller and Steinberg, 1977). We first describe increases in apical $[K^+]_o$ from this normal value, which is equivalent to the increase in $[K^+]_o$ in the intact retina upon return from light to dark, and then describe decreases in apical $[K^+]_o$.

A DELAYED BASAL DEPOLARIZATION Fig. 2 compares TEP and intracellular RPE apical and basal membrane responses from the isolated RPE-choroid of gecko and bullfrog evoked by increasing apical $[K^+]_o$ from 2 to 5 mM. In frog, this depolarized the apical membrane, i.e., produced a change in the apical

battery, as expected since this membrane has an appreciable K^+ conductance (Miller and Steinberg, 1977). The smaller depolarization of the basal membrane was the passive response caused by a change in the shunt current across the basal membrane. Since the apical membrane depolarization was larger than the basal depolarization, the TEP decreased.

In gecko, the response to an increase in apical $[K^+]_o$ was surprisingly different. The TEP response was biphasic; an initial decrease in TEP was followed by a

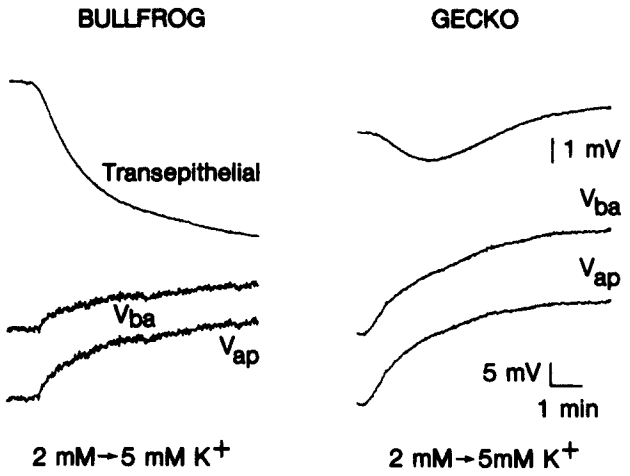

FIGURE 2. Comparison of responses from bullfrog and gecko. Preparation: isolated RPE choroid. The transepithelial potential (TEP) was recorded between the apical and basal bathing solutions; the apical membrane potential (V_{ap}) was recorded differentially between an intracellular microelectrode and the apical bath; the basal membrane potential (V_{ba}) was recorded differentially between the electrode and the basal bath. All potentials were recorded simultaneously while changing apical $[K^+]_o$ from 2 to 5 mM. Responses on the left are from the bullfrog; initially, TEP = 14.4 mV, $V_{ap} = -91$ mV, and $V_{ba} = -76$ mV. Responses on the right are from the gecko; initially, TEP = 9 mV, $V_{ap} = -95$ mV, and $V_{ba} = -86$ mV. In both frog and gecko, increasing apical $[K^+]_o$ depolarized both apical and basal membranes and initially decreased TEP. In the gecko, a delayed increase (rebound) of TEP was also present.

delayed increase (rebound) while the apical membrane was still being bathed in the 5 mM test solution. The TEP decrease and the delayed TEP increase were both accompanied by depolarizations of the RPE membranes. In frog, a change in apical $[K^+]_o$ never evoked a biphasic response.

Fig. 3 shows another set of responses from gecko to an increase in apical $[K^+]_o$, where the apical and basal membrane potentials have been superimposed before the response (*B*) and at the time the TEP response reverses polarity (*C*). During the initial decrease in TEP (period 1), the apical membrane depolarized relative to the basal membrane. We expected that this response, as in frog, was generated at the apical membrane by a change in the K^+ equilibrium potential across it (i.e., a depolarizing apical battery) and that the basal depolarization was

the shunted response. During the subsequent increase in TEP (period 2), both membranes continued to depolarize, but the basal membrane depolarized relative to the apical. Thus, in addition to the passive shunted depolarization across the basal membrane, there must have been a new depolarizing battery at the basal membrane that generated the delayed TEP increase. We call this new response the "delayed basal depolarization."

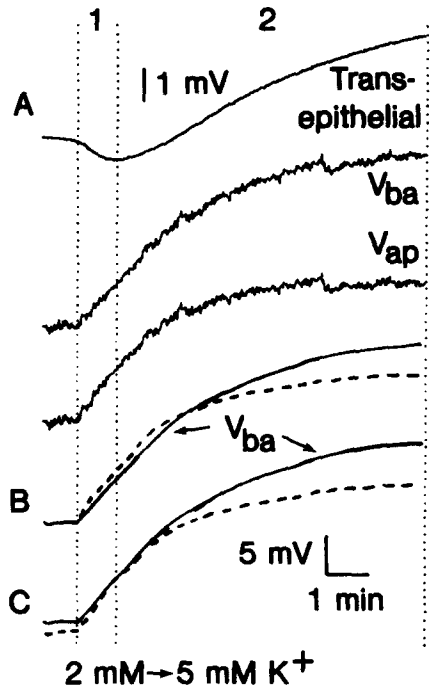

FIGURE 3. Responses of gecko isolated RPE-choroid to increased apical $[K^+]_o$. Transepithelial potential, V_{ap}, and V_{ba} were recorded simultaneously as in Fig. 2 while apical $[K^+]_o$ was switched from 2 to 5 mM. Initially, TEP = 17 mV, $V_{ap} = -84$ mV, and $V_{ba} = -67$ mV. In *B*, the apical and basal membrane potentials have been redrawn and superimposed before the response. In *C*, the membrane potentials have been redrawn and superimposed at the time that the TEP response reverses polarity. During period 1, the apical membrane depolarized more than the basal and the TEP decreased. During period 2, the basal membrane depolarized more than the apical, and the TEP increased.

We eliminated several alternative possibilities for the origin of the delayed TEP increase. If the paracellular shunt were permeable to K^+, then the potential produced at the shunt by increasing apical $[K^+]_o$ would be expected to decrease, not increase, the TEP. The delayed TEP increase, therefore, is not caused by a shunt battery. Increasing apical $[K^+]_o$ could have caused a junction potential between the agar-Ringer recording electrode (2 mM K^+) and the 5 mM K^+ test solution. A delayed junction potential would be unusual, however, and no delayed

TEP increase was observed with the bullfrog RPE-choroid under identical recording conditions. The increase of apical $[K^+]_o$ might have increased $[K^+]$ in the basal bath if K^+ leaked across the paracellular shunt or around the edge of the tissue, although the basal membrane was continually superfused with 2 mM K^+. Even in the presence of 5 mM K^+ in the basal bathing solution, however, a subsequent increase in apical $[K^+]_o$ from 2 to 5 mM produced the same TEP response as when the basal superfusate contained 2 mM K^+. We conclude that the new depolarization of the basal membrane originates there as a delayed response to the increase of apical $[K^+]_o$.

A DELAYED BASAL HYPERPOLARIZATION In the intact retina, light causes a decrease in subretinal $[K^+]_o$ that hyperpolarizes the cell (see Figs. 7 and 9). This can be mimicked in the isolated RPE-choroid by the return from 5 to 2 mM $[K^+]_o$ in the apical bath (Fig. 4). The initial TEP increase (period 1) was followed by a delayed decrease (period 2). Intracellular recordings show that during the increase in TEP, the apical membrane hyperpolarized more than the basal membrane, and that during the subsequent TEP decrease, the basal membrane hyperpolarized more than the apical. Thus, a decrease in apical $[K^+]_o$ from 5 to 2 mM caused a TEP increase generated by an apical hyperpolarization, followed by a delayed TEP decrease generated by a delayed basal hyperpolarization.

A decrease in apical $[K^+]_o$ from 5 to 2 mM is about the maximum change expected to occur in the subretinal space of an intact gecko retina in response to a light stimulus of high illuminance (see Fig. 7). Fig. 5 shows TEP responses evoked by smaller changes in apical $[K^+]_o$ within the physiological range. A 1-mM decrease in $[K^+]_o$ (from 3 to 2 mM) still produced the delayed basal response that caused a delayed decrease in TEP. A larger decrease in $[K^+]_o$, from 3 to 1 mM, produced both a larger increase in TEP (the apical response) and a larger delayed decrease in TEP. On the other hand, apical $[K^+]_o$ changes outside the physiological range, for example from 20 to 5 mM, produced only an increase in TEP (not shown).

RESISTANCE MEASUREMENTS To further characterize the delayed basal membrane responses in gecko, changes in membrane conductance were estimated by passing constant current pulses across the tissue while changing apical $[K^+]_o$. In most experiments current was passed in the basal to apical direction; current in the apical to basal direction gave similar results. Although individual RPE resistances were not measured, we could obtain the equivalent resistance of the RPE, R_t, and the ratio of apical to basal membrane resistance, a. For a given pulse of current, R_t is determined by the iR drop across the tissue, and a from the ratio of apical to basal cell membrane iR drops (Frömter, 1972; Miller and Steinberg, 1977). In the frog, changes of apical $[K^+]_o$ do not produce detectable changes in either R_t or a (Miller and Steinberg, 1977).

Fig. 6 shows the time course of changes in a and R_t produced by changing apical $[K^+]_o$ from 2 to 5 mM and then back from 5 to 2 mM; simultaneous recordings of TEP and intracellular basal membrane potential are also shown. As we have already shown in Fig. 3, increasing apical $[K^+]$ from 2 to 5 mM caused a decrease in TEP, generated by a depolarization of the apical membrane, followed by an increase in TEP, generated by a depolarization of the basal

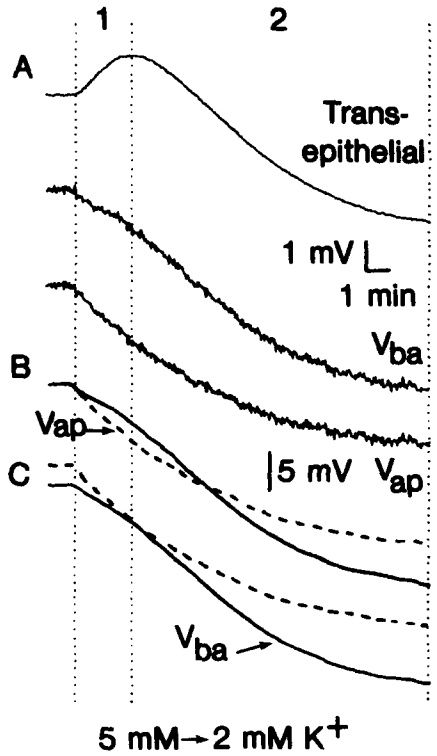

FIGURE 4. Response of RPE-choroid to decreased apical $[K^+]_o$. Transepithelial potential, V_{ap}, and V_{ba} were recorded from the same cell as in Fig. 3 while switching apical $[K^+]_o$ from 5 to 2 mM. Initially, TEP = 20 mV, V_{ap} = −66 mV, and V_{ba} = −46 mV. In *B*, V_{ap} and V_{ba} have been redrawn and superimposed before the response. In *C*, the membrane potentials have been redrawn and superimposed at the time that the TEP response reverses polarity. During period 1, the apical membrane hyperpolarized more than the basal, and the TEP increased. During period 2, the basal membrane hyperpolarized more than the apical, and TEP reversed and decreased.

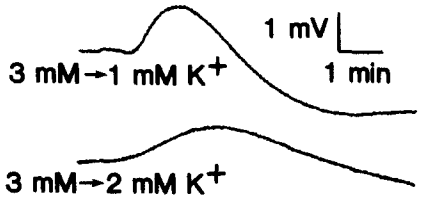

FIGURE 5. Transepithelial responses to a small change in apical $[K^+]_o$. Decreasing apical $[K^+]_o$ from 3 to 1 mM (top) or from 3 to 2 mM (bottom) produced an increase in TEP followed by a delayed TEP decrease. Initially, TEP = 17 mV (top) and 15 mV (bottom).

membrane. During the decrease in TEP (period 1), there was a small decrease in a and an increase in R_t. These small changes may result from movement artifacts when switching the solution or from a change in apical resistance (see footnote 1 below); in other examples, a decreased and R_t also decreased. Observe

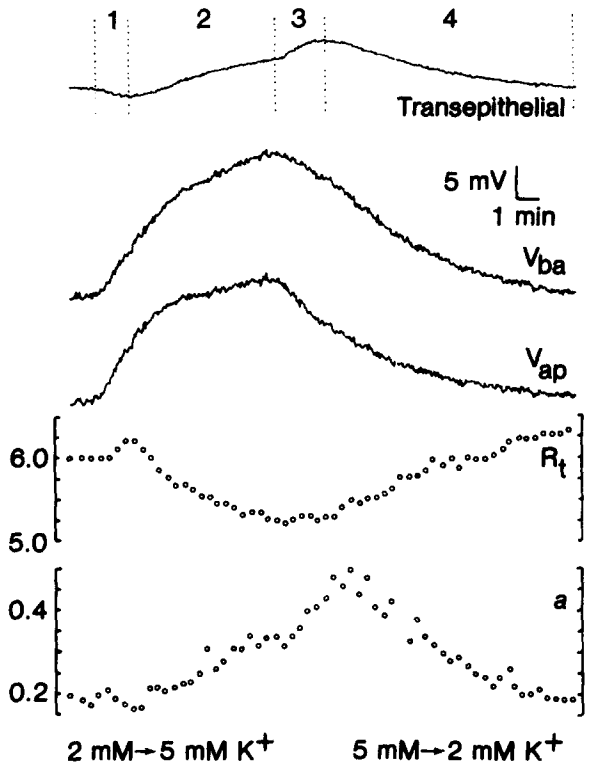

FIGURE 6. Resistance measurements. Transepithelial potential, V_{ap}, and V_{ba} were simultaneously recorded as in Fig. 2. Initially, TEP = 16 mV, V_{ap} = −80 mV, and V_{ba} = −64 mV. 2-μA pulses of current were passed across the tissue in the basal to apical direction. R_t (kΩ) was calculated from the iR drops across the tissue, and a is the ratio of the iR drops across the apical and basal cell membranes. The iR drops have been masked from the voltage responses. Below, the calculated values of R_t and a are plotted vs. time. When apical $[K^+]_o$ was switched from 2 to 5 mM, R_t decreased and a increased with the approximate time course of the delayed basal membrane depolarization and TEP increase. On return from 5 to 2 mM $[K^+]_o$, R_t and a both recovered, R_t increasing and a decreasing with the approximate time course of the delayed basal membrane hyperpolarization and TEP decrease.

that during the increase in TEP generated by the basal membrane (period 2), a increased from 0.2 to 0.33 and R_t decreased from 6.0 to 5.5 kΩ. This decrease in R_t indicates that at least one of the RPE resistances decreased, whereas an increase in a could result from an increase in apical resistance, a decrease in basal resistance, or both. Both the increase in a and the decrease in R_t could be explained by a decrease in basal resistance. Since we know that a basal membrane

depolarization generated the increase in TEP during period 2, it is tempting to conclude that the changes in R_t and a during this period are also generated at the basal membrane. We therefore hypothesize that the basal depolarization is accompanied by an increase in basal membrane conductance.

On returning from 5 to 2 mM $[K^+]_o$ in the apical bath, a TEP increase (period 3) was followed by a TEP decrease (period 4). During period 3, a continued to increase and there was no change in R_t.[1] During the TEP decrease generated by a hyperpolarization of the basal membrane, a decreased from 0.5 to 0.2 and R_t increased from 5.5 to 6.3 kΩ. Both the increase in R_t and the decrease in a during period 4 could be explained by an increase in the resistance of the basal membrane. Thus, an increase in apical $[K^+]_o$ leads to a delayed depolarization of the basal membrane and an apparent increase in basal membrane conductance; a decrease in apical $[K^+]_o$ leads to a delayed hyperpolarization of the basal membrane and an apparent decrease in basal conductance.

Light-evoked Responses

We next studied the effects of light in the retina-RPE-choroid preparation to investigate whether light-evoked changes in $[K^+]_o$ in the subretinal space would produce similar sequences of potential changes.

LIGHT-EVOKED CHANGES IN $[K^+]$ Light-evoked changes in apical $[K^+]_o$ were measured with a double-barreled K^+-selective microelectrode positioned in the subretinal space outside the RPE apical membrane. The potential between the K^+-selective barrel and the reference barrel is called V_{K^+} and is proportional to $[K^+]_o$ (Oakley, 1977). The TEP was measured simultaneously between the reference barrel and an electrode in the choroidal bathing solution. In the dark, the subretinal $[K^+]_o$ was between 3 and 5 mM ($n = 4$). Fig. 7 shows a series of V_{K^+} recordings in response to 3-min stimuli of increasing illuminances. At every illuminance, V_{K^+} transiently decreased and then returned to a plateau that was below the dark-adapted level. At the highest illuminance, the $[K^+]_o$ decreased from a dark level of 5 mM to a minimum of 3 mM and then reaccumulated to a plateau level of 3.5 mM. Thus, as in the frog (Oakley and Steinberg, 1982) and cat (Steinberg et al., 1980), light caused a transient decrease in apical $[K^+]_o$ to a minimum, followed by a reaccumulation to a plateau. When the light was turned off, $[K^+]_o$ overshot its dark-adapted baseline and then decayed. The magnitudes of the light-evoked changes in apical $[K^+]_o$ were similar to the increase and decrease in apical $[K^+]_o$ that produced the responses shown above for the isolated gecko RPE.

Fig. 8 compares V_{K^+} and TEP responses evoked by a 3-min step of illumination. The TEP first transiently decreased as a result of the passive iR drop of the b-wave current across the high resistance of the RPE. This current presumably returns to the retina via a path around the edge of the tissue, a pathway equivalent to the current that flows through the scleral resistance in the intact eye (Linsen-

[1] During period 3, we assume that the change back from 5 to 2 mM K^+ has not yet been signaled to the basal membrane, and therefore its resistance continues to decrease. Also, preliminary evidence suggests that the apical resistance may increase, and therefore a increases although R_t does not change.

meier and Steinberg, 1984; Rodieck, 1973). This was followed by an increase in potential and a delayed decrease while the light was maintained. We wanted to know whether the TEP increase and delayed decrease followed the decrease and subsequent reaccumulation of subretinal [K^+]. In frog V_{K^+} can be scaled to match the entire TEP response because the latter is determined by the response of the apical membrane to light-evoked changes in subretinal [K^+] (Oakley and Steinberg, 1982). In Fig. 8*B*, V_{K^+} has been inverted and scaled to the TEP response to facilitate comparison. V_{K^+} could be scaled to match the TEP increase, but it did not match the delayed TEP decrease; the TEP decreased more than was

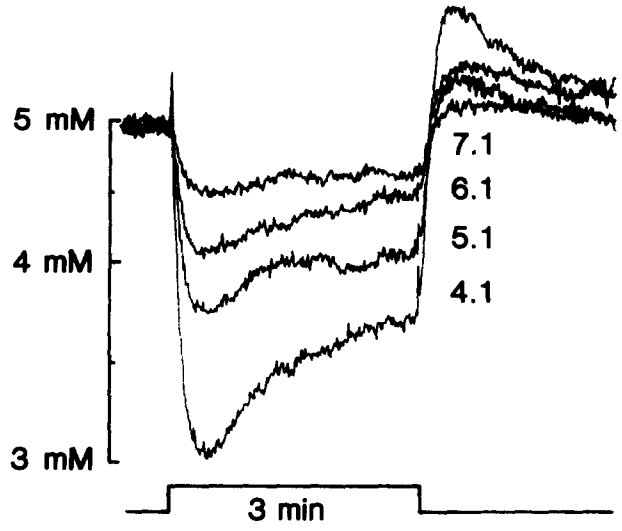

FIGURE 7. Light-evoked changes in subretinal $[K^+]_o$. A double-barreled K^+-selective electrode was positioned in the subretinal space; the difference in voltage between the K^+ and the reference barrel, termed V_{K^+}, is a measure of the $[K^+]_o$. V_{K^+} responses to 3-min stimuli of increasing illuminance are shown. For this tissue, the $[K^+]_o$ in the dark was ~5 mM, and at all illuminances, light caused a decrease in $[K^+]_o$ that reaccumulated to a plateau level during illumination. Numbers to the right of each record indicate log attenuation of the stimulus.

predicted by V_{K^+}. The failure of V_{K^+} to match the TEP decrease in gecko suggests that it is not generated solely by potential changes at the apical membrane, but could be caused by a delayed hyperpolarization of the basal membrane following the light-evoked decrease in apical $[K^+]_o$.

RPE INTRACELLULAR RECORDINGS To evaluate the changes in apical and basal membrane potentials that produced the TEP response to light, we recorded the TEP and the apical and basal membrane potentials simultaneously. A microelectrode was positioned extracellularly in the subretinal space between the neural retina and the RPE. By referencing this electrode to the choroidal bathing solution, we measured the TEP. To measure the RPE membrane potentials, a second microelectrode was positioned intracellularly within the RPE and refer-

enced to the choroidal bath to measure the basal membrane potential, V_{ba}. The apical membrane potential, V_{ap}, was obtained by subtracting V_{ba} from the simultaneously measured TEP.

Fig. 9*A* presents simultaneous TEP and intracellular RPE recordings in response to a 3-min stimulus. The TEP first increased to a peak at 30 s (period 1) and then decreased to a plateau above the dark-adapted level (period 2). The RPE apical membrane hyperpolarized rapidly for the first 30 s of illumination

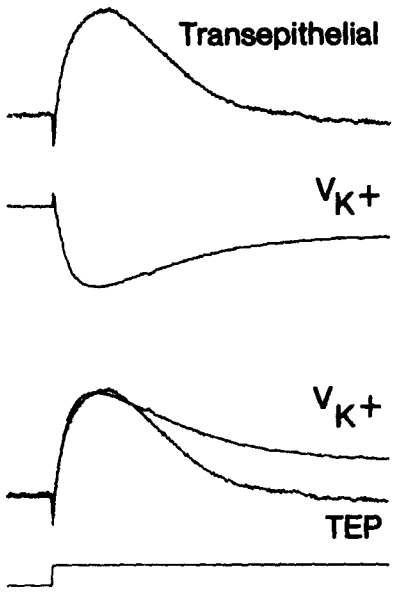

FIGURE 8. Comparison of TEP and V_{K^+}. A double-barreled K^+-selective electrode was positioned in the subretinal space. V_{K^+} was recorded as in Fig. 7; TEP was recorded differentially between the reference barrel of the electrode and the choroidal bathing solution. Responses to 3 min of illumination (attenuated by 0.1 neutral density [ND]) are shown. Initially, TEP = 11 mV; the peak TEP response is 3.52 mV above the baseline; the peak V_{K^+} response is 8.1 mV. Below, V_{K^+} was inverted and scaled to match the TEP response. Similar results were obtained with all stimulus attenuations used (0.1–6.1 ND).

and then continued to hyperpolarize more slowly for the duration of the stimulus; the basal membrane also hyperpolarized for the duration of the stimulus. In Fig. 9*B* the apical and basal membranes have been superimposed at the dark-adapted level. During the increase in TEP, the hyperpolarization of the apical membrane was greater than the basal hyperpolarization, and we can conclude that, as in the frog and isolated gecko RPE, the TEP increase is generated by a hyperpolarization of the apical membrane.

The delayed decrease in TEP from the peak to stimulus offset (period 2) was also accompanied by a hyperpolarization of both membranes. To compare the membranes during this period, apical and basal potentials have been superim-

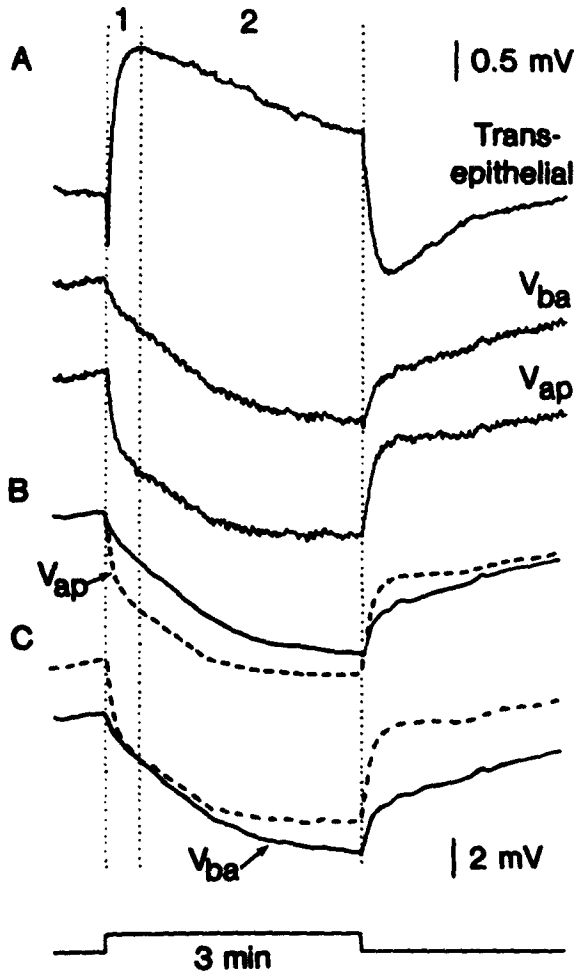

FIGURE 9. Light-evoked responses. Transepithelial potential (TEP) and apical (V_{ap}) and basal (V_{ba}) membrane potentials were simultaneously monitored with one electrode in the subretinal space and a second intracellular microelectrode in response to a 3-min step of illumination (attenuated by 4.1 ND). Initially, TEP = 11 mV, $V_{ap} = -88$ mV, and $V_{ba} = -77$ mV. In *B*, the V_{ap} and V_{ba} have been redrawn and superimposed in the dark; during period 1, the apical membrane hyperpolarized more than the basal. In *C*, V_{ap} and V_{ba} were redrawn and superimposed at the peak of the TEP increase. During period 2, the basal membrane hyperpolarized more than the apical. The upper calibration is for the TEP response; the lower is for the intracellular responses.

posed at the beginning of period 2, the TEP peak (Fig. 9*C*). During period 2, the basal membrane hyperpolarized relative to the apical membrane. Thus, during the decrease of TEP of period 2, a delayed hyperpolarization was generated at the basal membrane.[2]

When the light was turned off, an increase in K^+ (see Fig. 7) initiated a series of potentials of opposite polarity. The TEP first decreased since the apical membrane depolarized relative to the basal. Subsequently, the basal membrane depolarized more than the apical and TEP increased. This sequence at light offset is similar to that evoked in the isolated RPE-choroid by an increase in apical $[K^+]_o$ (see Figs. 4 and 5).

RESISTANCE MEASUREMENTS Changes in resistance were monitored by passing constant current pulses across the tissue and measuring the appropriate iR drops. Since the resistance of the neural retina was <10% of the trans-tissue resistance (retina plus RPE-choroid) and did not change in response to light, the iR drop across the tissue was used to monitor the transepithelial resistance, R_t. The ratio of the iR drop across the neural retina plus apical membrane to the iR drop across the basal membrane gave a very close approximation of a.

In gecko, light produced changes in both R_t and a. Fig. 10 shows a plot of R_t, a, and the simultaneously measured TEP and V_{ba} recordings. During the TEP increase (period 1), no changes in either R_t or a were observed. (It is possible that small changes may have occurred that went undetected; limits of detectability for a, ~0.05; for R_t, ~100 Ω.) During the subsequent TEP decrease (period 2), however, a decreased and R_t increased. Assuming that there were no changes in the shunt resistance, these results can be explained simply by an increase in basal membrane resistance. When the stimulus was turned off, a and R_t returned to their dark levels (not shown). Thus, the light-evoked changes in a and R_t are similar to those produced in the isolated gecko RPE by changing apical $[K^+]_o$ at the apical membrane. Although the changes in R_t and a were small, similar changes were recorded in all preparations from which such measurements could be made ($n = 4$).[3]

DISCUSSION

We have shown that in the isolated RPE-choroid of gecko, a change in apical $[K^+]_o$ first polarized the apical membrane, as expected for a membrane with an appreciable K^+ conductance, and then led, unexpectedly, to a delayed response

[2] Some gecko preparations exhibited a more complicated apical response. This was a biphasic increase in TEP at light onset; the TEP increased to a shoulder at ~20 s and reached a peak at ~60 s. It was accompanied by a biphasic hyperpolarization of the apical membrane and a smaller biphasic basal hyperpolarization. Both phases of TEP increase, therefore, were generated by hyperpolarizations of the apical membrane. A delayed TEP decrease, generated by a delayed hyperpolarization of the basal membrane, was still present. A complex apical response was not produced in the isolated RPE, and its origin in the intact retina-RPE-choroid preparation is under investigation.

[3] In preparations that responded to light with a biphasic apical hyperpolarization, an additional decrease in a accompanied by a decrease in R_t was observed. These resistance changes made it impossible to detect the small increase in R_t noted above.

of the basal membrane. This response was not due to a junction potential, a change in the shunt battery, or to leakage of K^+ into the basal bathing solution, but was generated by a potential at the basal membrane. Basal responses were produced by changes in $[K^+]_o$ as small as 1 mM and were present within the physiological range expected to be produced by light and dark in the intact retina.

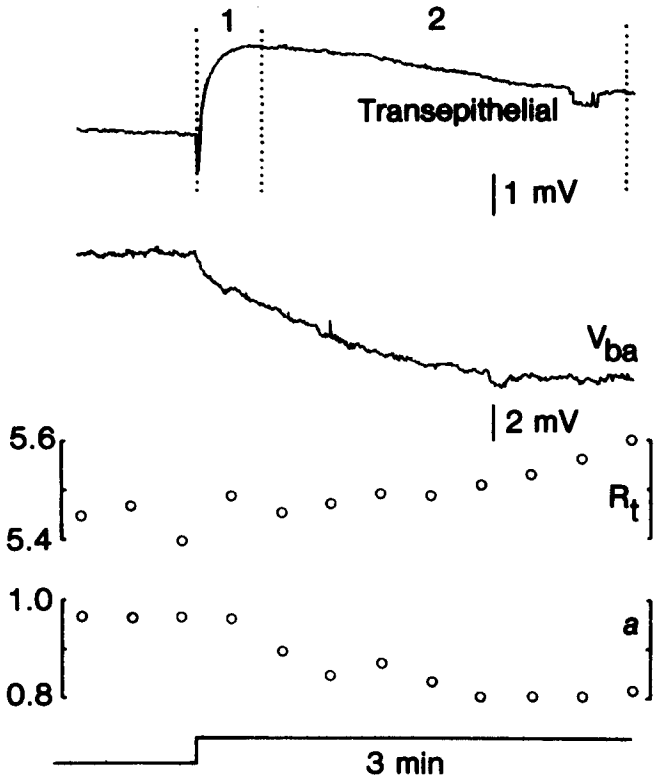

FIGURE 10. Light-evoked resistance changes. R_t (kΩ) and a were measured as in Fig. 6. Transepithelial potential and V_{ba} were recorded as in Fig. 9 (same cell, stimulus attenuated by 4.1 ND, TEP = 11 mV, and $V_{ba} = -77$ mV). Below, R_t and a are plotted vs. time. R_t increased and a decreased with the approximate time course similar to the delayed basal hyperpolarization and decrease in TEP from its peak. As in Fig. 6, the iR drops were masked from the voltage responses.

As predicted, light-evoked RPE responses from the intact gecko retina were qualitatively similar to those from the isolated RPE-choroid. At light onset, a decrease in $[K^+]_o$ produced an apical hyperpolarization and a TEP increase followed by a delayed basal hyperpolarization and a TEP decrease. At light offset, responses of the opposite polarity occurred. Thus, light-evoked changes in apical $[K^+]_o$ are signaled across the RPE to the basal membrane. A delayed basal response can be evoked by any dim and/or brief stimulus that decreases

apical $[K^+]_o$ (unpublished data; see also Linsenmeier and Steinberg, 1984). In contrast, the depolarizing response of the basal membrane during the light peak requires a stimulus of either long duration or high illuminance (Griff and Steinberg, 1982*a*; Linsenmeier and Steinberg, 1982). The neural retina thus has at least two mechanisms for signaling across the RPE to the basal membrane: one that is activated whenever apical $[K^+]_o$ changes, and a second slower mechanism that generates the light peak.

One difficulty in describing the new basal responses is the presence of potentials shunted to the basal membrane. In the isolated RPE-choroid, the delayed basal response evoked by changing apical $[K^+]_o$ is superimposed on a potential of the same polarity that is generated at the apical membrane and passively shunted to the basal membrane. Because shunted and generated potentials occur at both membranes in gecko, it is not simple to subtract the shunted apical polarization from the basal membrane potential to isolate the basal event alone (see Linsenmeier and Steinberg, 1984, for such an analysis in cat) and determine its time course. In the RPE-choroid preparation, however, the stimulus that evoked the delayed basal response was a step change in apical $[K^+]_o$. The TEP response reversed sign when the basal potential change (the sum of the delayed response generated at the basal membrane plus the shunted response) became larger than the apical potential (the sum of apically generated and shunted responses). Clearly, the delayed basal response had begun by the time the TEP reversed.

The light-evoked changes in the intact retina are more complex because apical $[K^+]_o$ decreased to a minimum and then reaccumulated to a plateau level. The initial $[K^+]_o$ decrease produced an apical hyperpolarization and, therefore, the TEP increased (Fig. 9). The decrease in TEP from its peak, however, was partly due to the recovery of the apical membrane potential following the recovery of $[K^+]_o$ in the subretinal space, and partly to the delayed basal hyperpolarization. One cannot see this apical repolarization (Fig. 9) because the hyperpolarization shunted from the basal membrane to the apical is larger than the apical repolarization itself. Significant shunting of a basal response to the apical membrane was also observed for the gecko light peak (Griff and Steinberg, 1982*a*). Thus, although we know that the delayed basal hyperpolarization is present after the TEP peak, we cannot determine when it begins.

At the end of 3 min of illumination, the TEP is still more positive than the dark-adapted level (Fig. 9), whereas the TEP response in the isolated RPE-choroid preparation evoked by a decrease in $[K^+]_o$ of similar magnitude is often below the baseline (Figs. 4 and 5). There are several reasons for the differences in the TEP responses. First, the time courses of the K^+ changes in the two preparations are different. In the retina-RPE-choroid preparation, the change is faster, reaching a transient minimum and then returning to a plateau level. The light-evoked decrease in K^+ also produces a retinal response, slow PIII, and the passive iR drop of slow PIII current across the RPE will increase the TEP (Linsenmeier and Steinberg, 1984). The light peak response, a depolarization of the basal membrane that increases TEP, may also start during the period of illumination (Griff and Steinberg, 1982*a*; Linsenmeier and Steinberg, 1982). Finally, the difference in TEP levels may reflect differences in the amount and

direction of shunting in the two preparations. In the retina-RPE-choroid, the value of a is relatively high,[4] so that more of the delayed basal response will be shunted to the apical membrane, and the resultant change in TEP will therefore be smaller (Linsenmeier and Steinberg, 1983).

In both preparations, we measured changes in R_t and a during the responses. The principal findings were that the delayed basal hyperpolarization was accompanied by an apparent decrease in basal membrane conductance and that the delayed basal depolarization was accompanied by an increase in conductance (Figs. 6 and 10). Other changes in resistance may also have occurred. In the isolated RPE-choroid preparation, the change in $[K^+]_o$ may alter the apical resistance before or during the delayed basal response (Fig. 6). In the retina-RPE-choroid preparation, the changes in R_t and a are small. The resistance changes associated with the delayed basal membrane hyperpolarization (Fig. 10) may be opposed by opposite changes that accompany the light peak (Griff and Steinberg, 1982*a*), if such changes begin within the first 3 min of illumination.

The mechanism of the delayed basal response in either preparation is as yet unknown. The K^+-dependent RPE basal responses evoked by light are similar to those produced by changing apical $[K^+]_o$ in the isolated RPE-choroid. This suggests that the delayed basal response evoked by light results specifically from the change in subretinal K^+ and not from other light-modulated substances or retinal potentials. Preliminary evidence indicates that a change in intracellular $[K^+]$ and/or $[Cl^-]$ follows the change in $[K^+]_o$ and that the intracellular change is required for the delayed basal response (Griff and Steinberg, 1982*b*). It is not known whether the basal responses are passive ionic changes, or whether they reflect modulations of an electrogenic pump, or whether the conductance changes cause the delayed basal responses or result from them (i.e., are voltage-sensitive conductances).

We can only speculate about the functional significance of these signals for the RPE. For example, since transport of substances in the choroid to retina direction must first occur at the basal membrane, these voltage changes may initiate or at least signal to us events that are involved in transport. It is, of course, quite likely that RPE transport would change in light and darkness, and therefore we should not be surprised to find that the basal membrane "knows" the level of retinal illumination and/or activity.

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[4] It is not known why a differs between the two preparations. Differences between the Ringer (RPE-choroid preparations) and the exact composition of fluid in the subretinal space may be one contributing factor.

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