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THREE LIGHT-EVOKED RESPONSES OF THE RETINAL PIGMENT EPITHELIUM

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Abstract—This paper summarizes our findings on light-evoked changes in retinal pigment epithelial cell (RPE) membrane potentials. Experiments were performed on the eye of the anesthetized or decerebrate cat and on isolated tissues from the eyes of a lizard, *Gekko gekko*, and a frog, *Rana catesbeiana*. In cat, as was previously shown, the RPE *apical* membrane potential responds to changes in $[K^+]_0$ in the subretinal space. At the onset of illumination it hyperpolarizes to a peak at 4.0 sec as $[K^+]_0$ decreases. The next RPE response is a hyperpolarization of the *basal* membrane that peaks at 20 sec and is also dependent on the decrease in subretinal $[K^+]_0$. The last and slowest response is a depolarization of the basal membrane that peaks at 300 sec, and is not obviously associated with K^+ changes. The same responses also appear in gecko at a slower time-course, but only the apical-membrane K^+-response is present in frog. The three responses also are associated with changes of the opposite polarity at the offset of illumination. These changes in membrane potential are the origin, respectively, of the RPE component of the ERG *c*-wave, the fast oscillation, and the light peak (slow oscillation).

Retinal pigment epithelium Retina Electroretinogram Electrooculogram Photoreceptors

INTRODUCTION

Retinal pigment epithelial (RPE) cells have two distinct cell membranes: a *basal* (basolateral) membrane facing the choroid and an *apical* membrane facing the photoreceptors across the subretinal space. Because the two membranes have different ionic properties and are electrically isolated to some extent by the tight junctions that physically separate them, they have separate membrane potentials (Miller and Steinberg, 1977). It has been known for some time that the apical membrane hyperpolarizes following absorption of light by photoreceptors and depolarizes with the onset of darkness (Steinberg *et al.*, 1970). This interaction between photoreceptors and RPE cells is mediated via changes in potassium ion concentration (Oakley and Green, 1976; Oakley *et al.*, 1977; Oakley, 1977), and resembles the interaction first described for neurons and glia (Kuffler and Nicholls, 1966).

The apical response to K^+ has been most completely studied in frog, where it is the only light-evoked electrical response of the RPE that can be found (Oakley *et al.*, 1977; Oakley, 1977; Oakley and Steinberg, 1982). The complex wave forms of the DC electroretinogram (DC-ERG) of reptiles, birds and mammals (Kikawada, 1968; Noell, 1953) and of human electrooculograms (EOG's), however, had suggested that additional RPE responses might be present in higher vertebrates. While other responses could be readily imagined for the apical membrane, it was initially difficult to consider that the basal membrane also could be a source of light-evoked, photoreceptor dependent, changes in membranes potential. The present paper summarizes our recent work in gecko, a lizard, and in cat that identifies two basal-membrane responses that follow the apical-K^+ response. The first, called the delayed basal hyperpolarization, also depends on the change in subretinal K^+ concentration ($[K^+]_0$) (Griff and Steinberg, 1984; Linsenmeier and Steinberg, 1984). For the second and much slower basal response, the light-peak depolarization, the molecule that mediates the interaction between the RPE cell and the neural retina remains to be determined (Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1982).

METHODS

The methods used for preparation and recording from the intact cat eye and from *in vitro* preparations of gecko have been reported previously and will be summarized only briefly here (Steinberg *et al.*, 1980; Linsenmeier and Steinberg, 1982, 1984; Griff and Steinberg, 1982, 1984).

We recorded from intact, urethane-anaesthetized or electrically decerebrated cats, paralyzed and artificially ventilated. Microelectrodes used for intraretinal and intracellular recordings were advanced into the eye through a hypodermic needle. Trans-epithelial recordings were made between a micro-electrode in the subretinal space and a reference in the orbit behind the eye (Fig. 1A). The basal membrane potential of RPE cells was recorded between an intracellular microelectrode and the reference behind the eye. The apical membrane potential was obtained from subtraction of a basal membrane response from a trans-epithelial response obtained subsequently. Potentials were amplified (DC to 50 Hz), displayed on a storage oscilloscope and chart recorder, and stored on magnetic tape. A PDP 11/03 computer was used later to digitize and plot the data. The stimuli were white light generated by either a fiberoptic illuminator and fiber

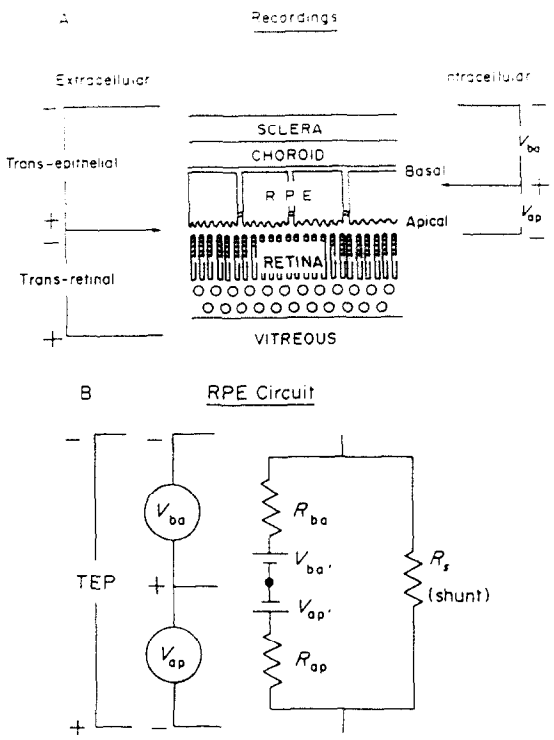

Fig. 1. (A) Diagram of recording configurations. As shown, for cat, the arrow (left) represents a microelectrode placed within the subretinal space that when referenced behind the eye recorded the trans-epithelial potential. Intracellular recordings were obtained by placing the microelectrode in the cytoplasm of an RPE cell (arrow, right). The basal membrane potential, V_{ba}, was recorded between this microelectrode and the reference behind the eye. The apical membrane potential, V_{ap}, was obtained from subtraction of a basal membrane response from a trans-epithelial response obtained subsequently. In gecko, the recording configurations were essentially the same, except that the apical and basal RPE membrane potentials were simultaneously recorded. (B) Schematic circuit of the retinal pigment epithelium. Circuit components: R_{ap}—apical membrane resistance; R_{ba}—basal membrane resistance; R_s—shunt resistance; V_{ap}'—apical membrane battery; V_{ba}'—basal membrane battery; V_{ap}—measured apical membrane potential; V_{ba}—measured basal membrane potential; TEP—trans-epithelial potential.

bundle, or by a dual beam ophthalmoscope. Diffuse illumination of the retina always was used. Retinal illumination for the cat is given in log quanta (507)-deg^{-2}-sec^{-1}, and in these units rod saturation is at about 8.2.

In the gecko (*Gekko gekko*) experiments animals were first refrigerated (10°C) to facilitate handling and then anesthetized by packing in ice. 5.0 mm square pieces of tissue, either neural retina, RPE and choroid, or only RPE and choroid were excised from the eye and mounted in a lucite chamber. In both preparations, the two sides of the tissue, retinal (or apical) and choroidal were continuously superfused by 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 $[K^+]_0$ (5.0 mM KCl) by means of a three-way valve.

The trans-epithelial and trans-retinal potentials were simultaneously recorded between a microelectrode in the subretinal space and agar-Ringer bridges in each of the baths (Fig. 1A). The apical and basal membrane potentials were obtained in a similar manner with an intracellular RPE microelectrode. Voltage drops across the tissue or across the RPE cell membranes were obtained by passing constant current pulses (1–5 μA for 1.0 sec) between agar-Ringer bridges located on each side of the tissue. Potassium-selective double-barrel microelectrodes were constructed, and calibrated as previously described (Oakley, 1977; Griff and Steinberg, 1984). The electrode was positioned in the subretinal space, just outside the apical membrane, to measure $[K^+]_0$. White light from a 100 W tungsten halogen lamp (3300 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. Data were analyzed as in the cat experiments.

RESULTS

Before describing our results it is important to review certain aspects of RPE structure and function.

RPE structure

Figure 2 diagrams the relevant structures in the retina. The RPE is a single layer of cells bounded proximally by the neural retina and distally by the choroid. Its basal surface rests on Bruch's membrane through which it communicates with the rich capillary bed of the choriocapillaris. Belts of tight junctions encircle the RPE cells dividing their membranes into basal (basolateral) and apical portions. The basal membranes and the tight junctions form the RPE portion of the barrier between the blood and neural retina. Entrance and exit of molecules to the neural retina can occur either across the two cell membranes and cytoplasm of the RPE, or across the tight junctions that form a *paracellular* route for movement (Miller and Steinberg, 1977). The apical membrane extrudes profuse villous and sheet-like processes that are closely apposed to and ensheath portions of the outer segments of the rods and cones.

The rod and cone cells protrude from the neural retina into the subretinal space, the primitive ventricular space, which is a larger space than any of the other extracellular spaces of the neural retina (Steinberg *et al.*, 1980). The subretinal space is bounded distally by the apical membranes of the RPE cells and proximally by the Müller cells and basal portions of the photoreceptor inner segments at the outer-limiting membrane. Sharing this space with the

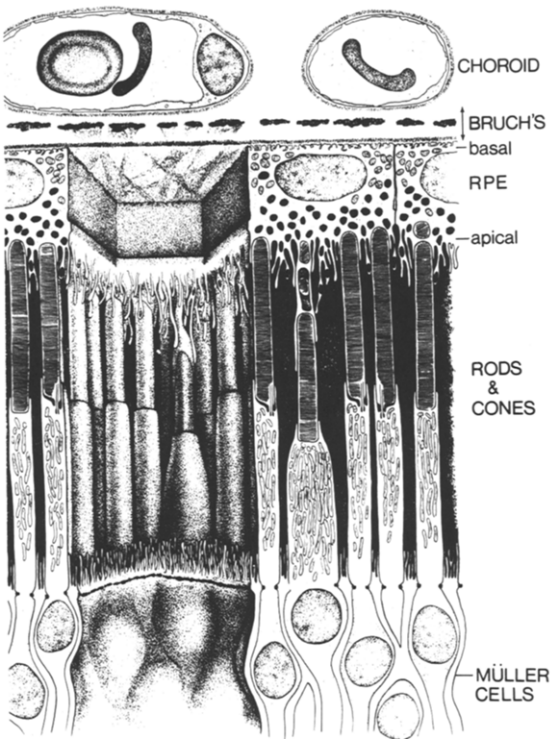

Fig. 2. Structure of the retina in the region of the subretinal space. The subretinal space is the extracellular space surrounding the photoreceptors (rods and cones). Its proximal (vitreal) boundaries are the basal portions of photoreceptor inner segments, and the Müller cells at the outer limiting membrane. Distally (sclerally) it is bounded by the apical membranes of RPE cells. The RPE is a single layer of cells bounded proximally by the neural retina and distally by the choroid. Its basal surface rests on Bruch's membrane. Belts of tight junctions encircle the RPE cells dividing their membranes into basal (basolateral) and apical portions. The basal membranes and the tight junctions form the RPE portion of the barrier between the blood and neural retina. The apical membrane extrudes profuse villous and sheet-like processes that are closely apposed to and ensheath portions of the outer segments of the rods and cones. This drawing generally follows retinal structure as observed in electron micrographs of human retina. Drawing by Joan Weddell.

photoreceptors are the apical processes of the RPE cells and the villous processes of the Müller cells.

RPE circuit

Figure 1(B) presents, in summary form, a circuit diagram of the RPE. More complete analyses of the RPE have been previously published and relevant equations can be found in those papers (Miller and Steinberg, 1977; Oakley, 1977; Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1983). A microelectrode placed intracellularly will record the apical membrane potential, V_{ap}, when referenced to the subretinal space, and the basal membrane potential, V_{ba}, when referenced to the back of the eye. The trans-epithelial potential, TEP, recorded between the subretinal space and the back of the eye, is the difference between V_{ap} and V_{ba}. These membrane potentials are not the same because of differences in the two membranes' passive and active ionic transport characteristics (Miller and Steinberg, 1977). In all the preparations so far examined, e.g. frog, lizard and cat, V_{ap} is more hyperpolarized than V_{ba} by at least 10 mV, so that the TEP is of this magnitude or larger and vitreal positive in sign.

If we are to interpret intracellular recordings, then we must take into account the effects of passive voltage drops at the cell membranes. Referring to the circuit diagram of Fig. 1(B), it will be clear that the apical and basal membranes are electrically connected through R_s (shunt resistance), primarily the resistance of the tight junctions between cells. Since the membrane potentials are different, a current flows around this circuit from the apical to basal membrane through R_s. This shunt current depolarizes the apical membrane potential from its theoretical maximum, V_{ap}'—the value that would be observed if R_s were infinite, and hyperpolarizes the basal membrane from its theoretical value, V_{ba}'. Most importantly, this current and the passive voltage drops resulting from it will change whenever a potential change is initiated at either cell membrane. The size and relative importance of this effect will vary depending on the relative size of all three resistances in the circuit—R_{ap}, the resistance of the apical membrane, R_{ba}, the resistance of the basal membrane and R_s. The practical effect of a change in shunt current is to reduce the magnitude of a voltage change at the membrane generating it and to produce a smaller voltage change of the same polarity at the opposite membrane. For example, a K^+ decrease in the subretinal space leads to a hyperpolarization that originates at the apical membrane. This causes a change in shunt current that produces a smaller, passive hyperpolarization of the basal membrane.

The apical membrane

The lower two records of Fig. 3 compare the response of the apical membrane with the light-evoked change in subretinal-space $[K^+]_0$ during 5 min of illumination of the cat retina. There is first a decrease of $[K^+]_0$ to a minimum at 4.0 sec, followed by a reaccumulation to a new steady-state level in the light. At the offset of illumination there is an overshoot of $[K^+]_0$ above the dark-adapted level and then a gradual return.

There is a remarkable correspondence between these changes in $[K^+]_0$ and the apical membrane response, V_{ap}—the apical membrane first hyperpolarizes to a peak as $[K^+]_0$ decreases and then repolarizes as $[K^+]_0$ reaccumulates. Parallel V_{ap} and $[K^+]_0$ events also occur at the offset of illumination. Essentially identical findings were also obtained in frog where it also has been shown that the changes of V_{ap} are solely due to the changes in the K^+ equilibrium potential at the apical membrane (Oakley, 1977; Miller and Steinberg, 1977; Oakley and Steinberg, 1982). In both cat and frog the apical membrane responses to K^+ are the origin of the RPE component of the ERG *c*-wave.

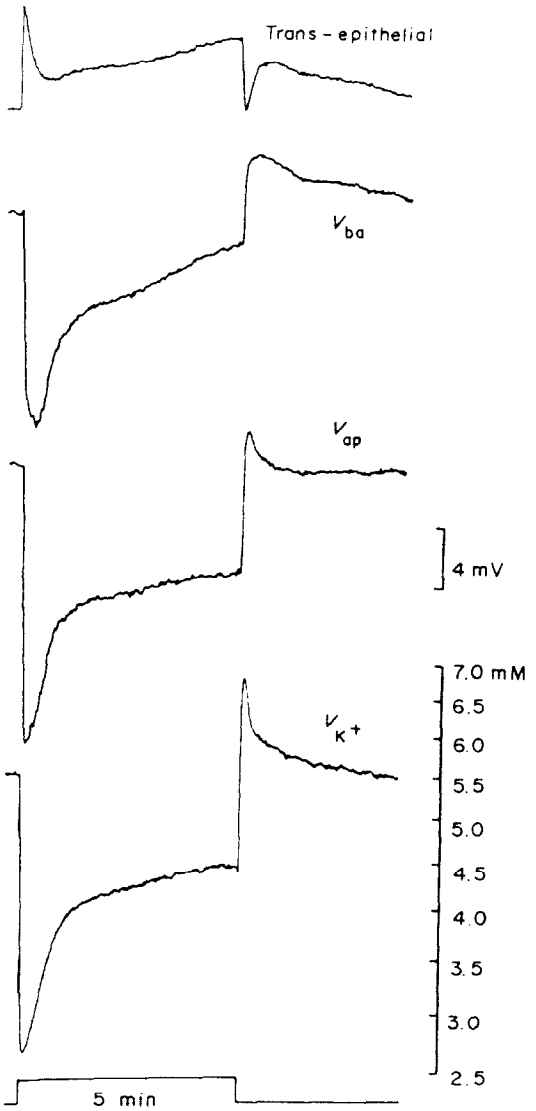

Fig. 3. Light-evoked responses in cat to 5 min of illumination: trans-epithelial potential, basal membrane potential (V_{ba}), apical membrane potential (V_{ap}) and subretinal potassium concentration (V_{K^+}). For the top three traces illumination was diffuse white light, 9.3 log q-deg^{-2}-sec^{-1} (from Linsenmeier and Steinberg, 1982). For the V_{K^+} trace illumination was a large spot (75 deg^2), 440 nm, 8.1 log q-deg^{-2}-sec^{-1} (from Steinberg *et al.*, 1980).

The basal membrane

Although the apical membrane follows subretinal $[K^+]_o$, the TEP, which is the sum of apical and basal responses, exhibits a more complex time-course (top, Fig. 3). This additional complexity results from the presence of two distinct responses of the basal membrane that appear in the V_{ba} recording of Fig. 3. The first basal response, called the delayed basal hyperpolarization, makes the initial hyperpolarization and subsequent repolarization of V_{ba} slower than the V_{ap} response. The second basal response, called the light-peak depolarization, produces a very slow depolarization of V_{ba} during the continuation of illumination.

Delayed basal hyperpolarization. The responses that originate at the basal membrane are combined in Fig. 3 with the change in V_{ba} that is due to shunting of the V_{ap} response. (In cat, the relative resistances of the membranes are such that there is little or no shunting of basal-membrane responses to the apical membrane). Thus, in order to describe responses that originate basally it is helpful to isolate them from the passive voltage drop due to shunting of the apical response. This was accomplished by estimating the basal response that was due to shunting alone, as a constant fraction of the apical response. (For further details see Linsenmeier and Steinberg, 1984.) Figure 4 presents the initial 135 sec of the response to a 5 min stimulus, showing the shunted response (dashed line) superposed on the actual apical and basal responses. Observe that the peak hyperpolarization of V_{ba} occurs later than that of V_{ap}, and that after the initial 3–4 sec, V_{ba} departs significantly from the response expected

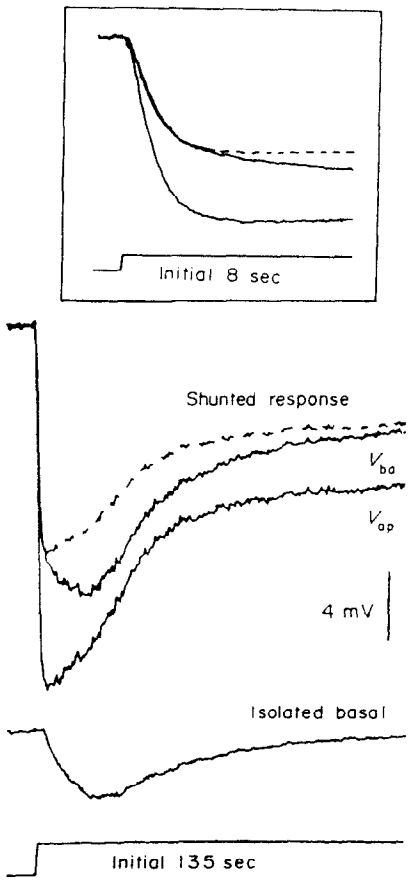

Fig. 4. Isolation of the delayed basal hyperpolarization in cat. The initial 135 sec of responses to 5 min of illumination are shown. Intracellular recordings of the apical (V_{ap}) and basal (V_{ba}) membrane potentials are superposed with an estimate of the shunted response at the basal membrane (interrupted line). Subtraction of the shunted response from the basal response gave the isolated basal response (bottom trace). The *inset* (top) shows the initial 8 sec of the responses at one-half gain, demonstrating that the shunted response accounts for V_{ba} over the initial 3–4 sec of the response. Illumination at 9.3 log q-deg^{-2}-sec^{-1} (from Linsenmeier and Steinberg, 1984).

due to shunting (inset, Fig. 4). By subtracting the shunted response from V_{ba} we obtain an estimate of events originating at the basal membrane (bottom trace, Fig. 4). This *delayed hyperpolarization* has a much slower time-course than the apical response to $[K^+]_o$, peaking at about 20 sec after the onset of illumination, and then slowly repolarizing to the dark-adapted level. The delayed hyperpolarization is the origin of the fast oscillation trough seen between the *c*-wave and light peak during maintained illumination (Linsenmeier and Steinberg, 1984).

This response also can be studied, *in vitro*, in the isolated retina-RPE-choroid of gecko. Figure 5 presents intracellular RPE recordings and a subretinal $[K^+]_o$ response from another experiment to a 3 min stimulus. In gecko, in contrast to cat, V_{ap} also includes a passive voltage drop from potentials generated at the basal membrane, and so V_{ap} does not exactly correspond to subretinal $[K^+]_o$ for the entire duration of the response. Superposition of V_{ap} and V_{ba} shows that during period 1 V_{ap} hyperpolarizes relative to V_{ba}, as expected for the change in E_K that hyperpolarizes the apical membrane. The continuing hyperpolarization of the apical membrane that follows is, however, not expected because $[K^+]_o$ actually is *increasing* during this period. The superposed responses show that during period 2 the basal membrane hyperpolarizes relative to the apical membrane. Thus, a delayed basal hyperpolarization also is present in gecko.

It is possible to mimic the light-evoked decrease in

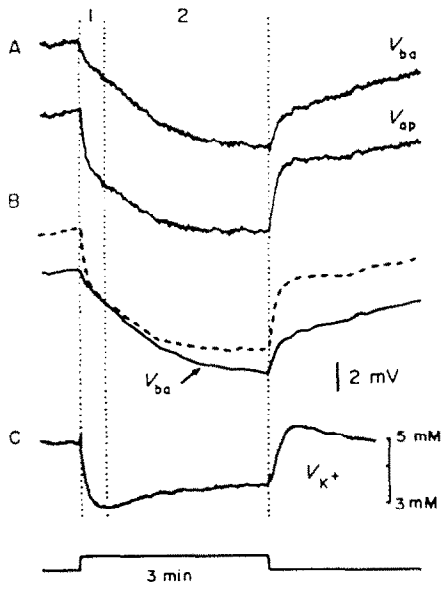

Fig. 5. Light-evoked responses in gecko to 3 min of illumination. A. Intracellular recordings of the apical (V_{ap}) and basal (V_{ba}) membrane potentials. (B) Superposition of V_{ap} (interrupted line) and V_{ba} to show that during period 2, V_{ba} hyperpolarized more than V_{ap}. (C) Light-evoked change in subretinal potassium concentration (V_{K^+}), from another experiment. The fast transient at on and off of light are artifactual. Illumination at −4.1 log attenuation for all responses (from Griff and Steinberg, 1984).

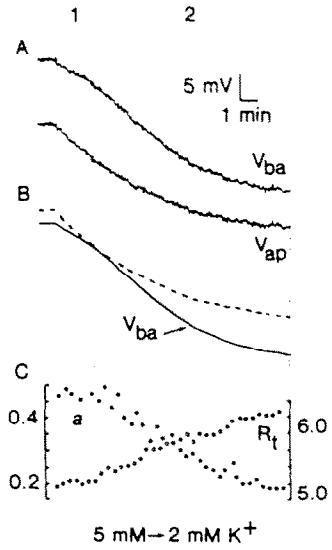

Fig. 6. Responses of gecko isolated RPE-choroid to a decrease in apical $[K^+]_o$. (A) Apical (V_{ap}) and basal (V_{ba}) membrane potentials in response to switching from 5 to 2 mM $[K^+]_o$. (B) Superposition of V_{ba} and V_{ap} (interrupted line) to show that during period 2 V_{ba} hyperpolarized more than V_{ap}. (C) Resistance measurements. In another experiment V_{ap} and V_{ba} were simultaneously recorded as in (A). Pulses of 2 μA current were passed across the tissue. R_t was calculated from the iR drops across the tissue, while a is the ratio of the iR drops across the apical and basal cell membranes (R_{ap}/R_{ba}). The calculated values of R_t and a are plotted against time (from Griff and Steinberg, 1984).

$[K^+]_o$ in a preparation of isolated gecko RPE-choroid, i.e. in the absence of the neural retina. In the experiment of Fig. 6, $[K^+]_o$ in the solution bathing the apical membrane was first increased from 2 to 5 mM and then, as shown, decreased from 5 to 2 mM. The latter change was about the maximum produced by light in the subretinal space. Superpositon of V_{ap} and V_{ba} shows that decreasing $[K^+]_o$ to 2 mM first hyperpolarized the apical membrane relative to the basal membrane during period 1. This is the response expected at the apical membrane for the change in E_K and is the only response observed in the frog (Oakley, 1977). In the gecko, however, V_{ba} subsequently hyperpolarized relative to V_{ap} (period 2). Thus, the change from 5 to 2 mM K^+ at the apical membrane initiated a delayed hyperpolarization that originated at the basal membrane; the same response that was produced by light.

To further characterize the delayed basal-membrane response, changes in membrane conductance were estimated by passing constant current pulses across the tissue while changing apical $[K^+]_o$. Although individual RPE resistances were not measured, we could obtain the resistance ratios R_t, the equivalent resistance of the RPE, and a, the ratio of the apical to basal membrane resistance. 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 changes in either R_t or a (Miller and Steinberg, 1977).

The time-course of changes in a and R_t produced by changing apical $[K^+]_o$ from 5 to 2 mM are shown at the bottom of Fig. 6. Observe that during the delayed basal hyperpolarization R_t increases from 5.2 to 6.2 KΩ and a decreases from 0.4 to 0.2. This increase in R_t indicates that at least one of the RPE resistances increased, while the decrease in a could result from a decrease in apical resistance, an increase in basal resistance, or both. Both the increase in R_t and the decrease in a could be explained by an increase in basal resistance. We, therefore, have hypothesized that the basal hyperpolarization is accompanied by a decrease in basal membrane conductance.

Light-peak depolarization. In Fig. 7, V_{ba} and V_{ap} from the cat are shown for the first 8 min of the response to 10 min of illumination. As we have seen in Fig. 3 the apical membrane repolarizes to a new steady-state level during maintained illumination that is still more hyperpolarized than the dark-adapted level; and this follows the reaccumulation of subretinal $[K^+]_o$. Superposition of the shunted response, the dashed line in Fig. 3, on V_{ba} shows that the basal membrane undergoes a slow depolarization that appears to begin at about 2 min (arrow) and peaks at 5 min after the onset of illumination. This response is termed the light-peak depolarization because it is the origin of the first light-evoked slow oscillation of the corneoretinal potential—the light peak (Täumer, 1976). The entire sequence of light-evoked membrane responses are shown in the isolated basal response at the bottom of Fig. 7. The basal membrane first hyperpolarizes and then repolarizes back to the dark-adapted level—the delayed basal hyperpolarization. This is followed by a much slower depolarization and repolarization—the *light peak*.

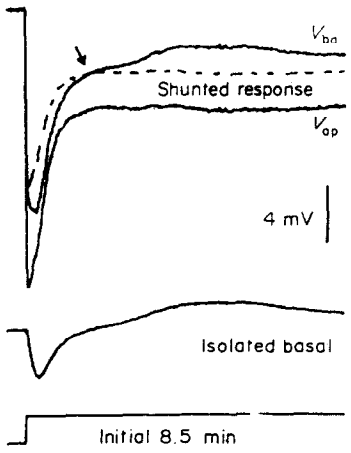

Fig. 7. Isolation of the light-peak depolarization in cat. The initial 8.5 min of responses to 10 min of illumination are shown. Format as in Fig. 4. The arrow at 2 min indicates the approximate time at which the basal membrane begins to depolarize relative to the apical. Subtraction of the shunted response from the basal response shows the delayed basal hyperpolarization followed by the light-peak depolarization. Illumination at 9.3 log q-deg^{-2}-sec^{-1}.

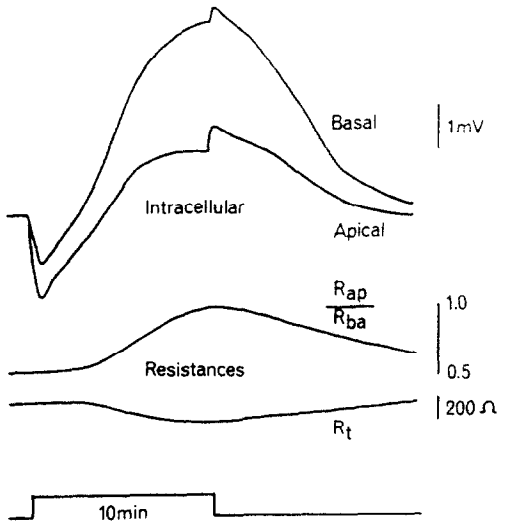

Fig. 8. The light peak in gecko in response to 10 min of illumination. Intracellular recordings (top) of the apical and basal membrane show that the light peak originates as a depolarization of the basal membrane. Because of shunting the apical membrane also depolarizes with the same time-course. Resistance measurements (bottom) as in Fig. 6 show the time-course of changes in a (R_{ap}/R_{ba}) and R_t. Illumination at 0.1 log attenuation for all responses (redrawn from Griff and Steinberg, 1982).

The light-peak depolarization was also observed in gecko, as shown in Fig. 8. In response to 10 min of illumination there was a large depolarization of the basal membrane relative to the apical membrane that peaked at 10 min in this cold-blooded vertebrate. Figure 8 also demonstrates that both the transepithelial resistance R_t, and the ratio a, change with the time-course of the light-peak depolarization. Following our interpretation for resistance changes given above, the changes during the light peak are most consistent with an increase in basal-membrane conductance.

Responses to the offset of illumination

In general, the RPE apical and basal membrane responses have been shown to undergo changes at the offset of illumination that are opposite in polarity to those that are caused by the onset of illumination. Briefly, for the apical membrane, as described in Fig. 3, a depolarization is observed during the overshoot of subretinal K^+. For the basal membrane, a delayed depolarization is observed at the offset of illumination, which is the equivalent of the delayed hyperpolarization. In the cat this response is most clearly observed at the termination of shorter flashes (about 1 min) that do not produce a prominent light-peak depolarization. As expected, experiments in the gecko isolated RPE-choroid preparation have shown that the delayed depolarization can be produced by an increase in $[K^+]_o$ at the apical membrane (2–5 mM) and is accompanied by resistance changes that are compatible

with an increase in basal-membrane conductance. We have not studied the equivalent off-response of the light-peak. It is known that there is a decline in the corneoretinal potential, the dark trough, which is initiated by the offset of illumination (Arden and Kelsey, 1962). We hypothesize that the dark trough is generated by a very slow hyperpolarization of the basal membrane.

DISCUSSION

Illumination initiates a sequence of three membrane-potential changes involving both RPE membranes and continuing for minutes after light onset. The three responses peak at progressively longer latencies—4, 20 and 300 sec (cat), and exhibit progressively slower time constants (Fig. 9). Although the RPE responses are slow, illumination does not have to be maintained for minutes to elicit them. Duration vs intensity trade-offs exist for each response and the entire sequence can be elicited by an intense flash lasting only 10 sec (Linsenmeier and Steinberg, 1982). The first two events clearly depend on photoreceptor-initiated changes in subretinal $[K^+]_o$ and the best available evidence indicates that the light-peak depolarization also is photoreceptor dependent (Griff and Steinberg, unpublished observation). Light offset initiates a repetition of the sequence but with the responses having the opposite polarity from light onset. Eventually we would like to know if these membrane-potential effects are indicative of modifications of RPE cellular function that occur as the needs of the photoreceptors change with illumination. To answer this question for each response we must know more about its mechanism of generation.

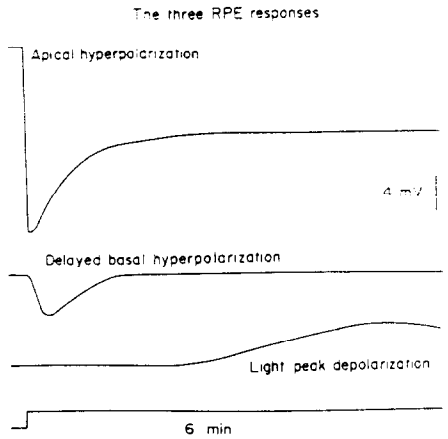

Fig. 9. Diagrammatic summary of the three light-evoked responses showing their relative time-courses and amplitudes. The initial 6 min of the response to maintained illumination are shown. The first response (top) is a hyperpolarization of the apical membrane that reaches its maximum at 4 sec. This is followed by two responses of the basal membrane: the delayed basal hyperpolarization, peaking at 20 sec and the light-peak depolarization reaching its peak at 300 sec. The offset of illumination produces a similar sequence of responses but of the opposite polarity (not shown).

Response mechanisms

Figure 10 presents a diagramatic summary of current knowledge. We know that the apical membrane has a significant K^+ conductance (Miller and Steinberg, 1977), that $[K^+]_o$ changes as a function of light and darkness in the subretinal space, and, therefore, that the change in apical-membrane potential represents the change in E_K at the apical membrane (Oakley, 1977). We do not yet know, however, for any RPE other than bullfrog whether there exist additional effects of K^+ on the apical membrane, such as changes in membrane conductance. We also have insufficient knowledge about how K^+ is regulated in the subretinal space to produce the specific sequence of $[K^+]_o$ changes that occur after the onset and offset of illumination. What, for example, are the comparative roles of

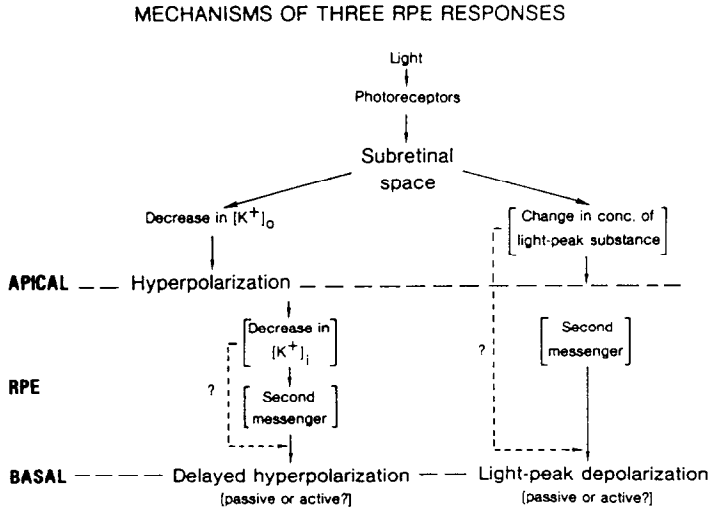

Fig. 10. Mechanisms of the three RPE responses. The sequence of steps leading to each response are shown. Steps in brackets have yet to be demonstrated. Interrupted lines indicate alternative pathways.

diffusion and ion pumps in the various cells surrounding the subretinal space, and of changes in the time-course of the photoreceptor light response, in producing the time-course of the $[K^+]_0$ change that occurs during maintained illumination (see Oakley, 1983 this issue).

The first step towards understanding the mechanism of the delayed basal hyperpolarization was the establishment of its dependency on subretinal $[K^+]_0$ using the isolated choroid-RPE preparation. There must then be one or more intracellular steps in a sequence of events that lead to the change in basal membrane potential; and the latter may result from a change in ionic conductance or ion pump rate. Experiments, to be reported elsewhere (Griff and Steinberg, in preparation), suggest that one event in the sequence may be a change in the intracellular K^+ concentration that follows the change in subretinal $[K^+]_0$, and that the basal response may be generated by a change in membrane conductance. We do not yet know whether the apparent time-related change in basal-membrane conductance that we have measured represents this conductance change or whether it is a voltage-sensitive response. We anticipate that more can be learned about this response using the *in vitro* RPE-choroid model.

The light-peak depolarization remains the most mysterious of the three responses. It does not, because of its time-course, appear to be directly associated with the $[K^+]_0$ changes of the subretinal space or elsewhere in the neural retina (Steinberg and Niemeyer, 1981). We have postulated that some other substance is produced (or consumed) in the retina in response to light, and that this substance diffuses to (or away from) the apical membrane of the RPE (Linsenmeier and Steinberg, 1982). The effect of this substance or that of interposed intracellular messengers on the basal membrane is not known and, again, we do not yet know whether the observed change in basal membrane resistance is causal or a consequence of the light-peak depolarization.

Corneoretinal potential

Each of the three RPE changes in membrane potential contributes to changes in the corneoretinal potential recorded in the human DC-ERG or EOG (Skoog, 1975; Täumer, 1976). For the *c*-wave alone, there also is a retinal component, slow PIII, of similar time-course, that contributes substantially to the DC-ERG recordings (Faber, 1969; Rodieck, 1972). But the principal origins of the fast oscillation (Kolder and Brecher, 1976; Kolder and North, 1976) are the delayed basal membrane hyperpolarizing and depolarizing events; and the light-peak originates solely from the very slow depolarization of the basal membrane.

The origin of each ERG response has been considered in detail elsewhere (Linsenmeier and Steinberg, 1982; 1983; 1984) but one additional point can be made concerning the relative sizes of the membrane-potential changes and the ERG. Quite small changes in basal membrane potential can be recorded faithfully at the cornea. The fast oscillation and light peak, for example, represent changes in membrane potential of only about 5 mV or less (Fig. 9). In cat, it is the absence of significant shunting from the basal to the apical membrane that allows these responses to be recorded in the vitreous at near their full magnitude. Another factor is the absence of a retinal component that subtracts from the RPE response. For the *c*-wave, however, both shunting (apical to basal) and the presence of slow PIII act to substantially diminish the final magnitude of the ERG *c*-wave, even though this response at the apical membrane can be as large as 20 mV (Fig. 9). At the cornea, in human, or vitreous, in cat, the result is that the light peak can be considerably larger than the *c*-wave. This similarity in human and cat suggests that the mechanisms of generation, including the relative resistances of the RPE apical and basal membranes, also may be similar.

Photoreceptor RPE interaction

We can imagine that the three responses described above may be involved in any of the functional interactions between RPE cells and photoreceptors. The retinal pigment epithelial cell can be thought of as comprising three distinct cell types with regard to its major functional interactions with the photoreceptors (Fig. 11). As a *macrophage* it daily and nightly phagocytoses the shed outer-segment tips of photoreceptors (for a review see Besharse, 1982). As an *epithelial cell* it transports substances to and from the blood and subretinal space (Steinberg and Miller, 1979). More speculatively, as a *glial cell*, it ensheaths the outer segments, responds to K^+ in a glial manner, participates in regulating the ion and water content of the subretinal space (Miller and Steinberg, 1982; Miller *et al.*, 1982), and may do whatever else, in the future, glial cells are found to do with respect to neurons.

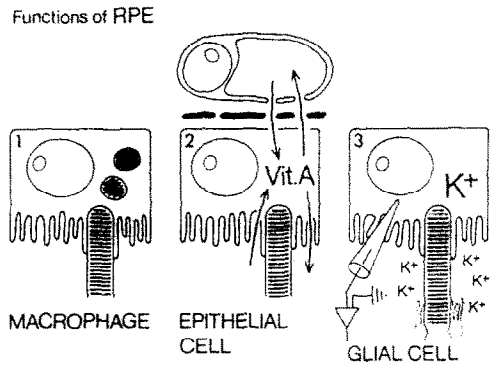

Fig. 11. Functions of the RPE. The RPE can be viewed as functioning as three distinct cells: (1) as a *macrophage* it phagocytoses and degrades the shed tips of photoreceptor outer segments, (2) as an *epithelial cell* it transports molecules between the blood and the subretinal space, (3) as a *glial cell*, its apical membrane ensheaths portions of the photoreceptor outer segments and responds to changes in subretinal $[K^+]_0$.

A change in membrane potential, itself, may have functional significance, for example, by affecting a voltage-sensitive ion conductance. It is even more likely, however, that in non-excitable cells such as the RPE (Peterson, 1980; Williams, 1981) the cytoplasmic or membrane events underlying the change are significant for cell function. For the apical membrane there are no conductance changes, at least in frog, but we do know that the change in subretinal $[K^+]_0$ alters the rate of pumps on the apical membrane that are K^+-sensitive, and thereby modulates ion and metabolite transport across this membrane (Miller and Steinberg, 1979, 1982). For the basal membrane the ionic events underlying the two responses must, themselves, consist of changes in ion concentrations, permeabilities or pump rates. It is easy to imagine, therefore, that accompanying these changes in membrane potential are changes in ion, metabolite or water transport between the RPE and the blood. It is exciting to consider that changes of the concentration of substances in the subretinal space, which are themselves the consequences of neural retinal and primarily photoreceptor activity, reach across the RPE cell to modify the function of the basal membrane.

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