Potassium and the Photoreceptor-Dependent Pigment Epithelial Hyperpolarization

BURKS OAKLEY II

From the Department of Physiology, University of California, San Francisco, California 94143

ABSTRACT Light-evoked changes in pigment epithelial cell membrane potentials and retinal extracellular potassium ion concentration, [K+]0, were measured in an in vitro frog retina-pigment epithelium-choroid preparation. Light stimuli hyperpolarized the apical membrane of the pigment epithelium. Through an electrical shunt pathway connecting the apical and basal membranes, the basal membrane also hyperpolarized, but to a lesser degree than the apical membrane. This differential hyperpolarization of the two membranes increased the transepithelial potential (TEP). This increase in TEP was shown to be the major voltage source of the c-wave of the electroretinogram (ERG). Direct measurement of [K+]0 in the distal retina, made with K+-specific microelectrodes, showed a light-evoked decrease in [K+]0 having an identical time course to the apical hyperpolarization. There was a linear relationship between the light-evoked change in TEP and the logarithm of [K+]0. This exact relationship was also found when the apical membrane was perfused directly with solutions of varying [K+]0. The change in TEP associated with the ERG c-wave, therefore, was explained solely by the response of the pigment epithelium to the light-evoked decrease in [K+]0 in the distal retina.

INTRODUCTION

The c-wave of the electroretinogram (ERG) is believed to be generated primarily by the pigment epithelium (Noell, 1954, 1963; Brown and Wiesel, 1961; Steinberg et al., 1970), which is a single layer of epithelial cells located between the neural retina and the choroid. Intracellular recordings from cat pigment epithelial cells in vivo have shown that the apical membrane (the membrane facing the photoreceptors) responds to a light stimulus with a slow hyperpolarization, whose time course is the same as that of the c-wave (Steinberg et al., 1970). This hyperpolarization is dependent upon the absorption of light by the photoreceptors since it has the rod action spectrum (Steinberg et al., 1970) and cannot be measured from the isolated pigment epithelium (Brown and Crawford, 1967). It has been suggested that this hyperpolarization is caused by a light-evoked change in the extracellular concentration of an ion (Noell, 1954, 1963; Steinberg et al., 1970; Steinberg and Miller, 1973). It is very likely that this ion is potassium since, in the frog, the apical membrane of the pigment epithelium has a high relative potassium conductance (Miller and...
Steinberg, 1977) and there is a light-evoked decrease in extracellular potassium ion concentration \([K^+]_0\) at the retinal side of the pigment epithelium, with a time course similar to that of the c-wave (Oakley and Green, 1976). On the basis of these observations, it seems possible that the light-evoked hyperpolarization of the apical membrane is produced solely by a change in \([K^+]_0\).

There are, however, several other mechanisms which may contribute to the generation of the pigment epithelial hyperpolarization. The apical membrane of the frog pigment epithelium has an appreciable bicarbonate conductance; a light-evoked increase in extracellular bicarbonate ion concentration \([HCO_3^-]_0\) could therefore contribute to the pigment epithelial hyperpolarization (Steinberg and Miller, 1973). There could also be light-evoked changes in the concentrations of other ions or molecules which could alter the apical membrane conductance to potassium and/or bicarbonate, and thus influence the magnitude of the pigment epithelial hyperpolarization. In addition, changes in the rate of an electrogenic pump on the apical membrane, due to the ion concentration changes, could influence the magnitude of the hyperpolarization.

In the experiments reported in this paper, the light-evoked pigment epithelial hyperpolarization and the c-wave were studied in an in vitro frog retina-pigment epithelium-choroid preparation. Intracellular microelectrodes were used to characterize the hyperpolarization, and potassium-specific microelectrodes were used to quantify the light-evoked decrease in \([K^+]_0\). There was no change in apical membrane conductance during the hyperpolarization. The membrane hyperpolarization increased the transepithelial potential (TEP) with the same time course as the ERG c-wave. The increase in TEP accounted for the major fraction of the c-wave voltage. There was a linear relationship between the change in TEP and the logarithm of \([K^+]_0\). This exact relationship was also found in an isolated pigment epithelium-choroid preparation when \([K^+]_0\) was varied in the solution perfusing the apical membrane. Therefore, the change in transepithelial potential associated with the c-wave seems to be caused solely by the light-evoked decrease in \([K^+]_0\).

**Materials and Methods**

**Preparation**

Large bullfrogs *Rana catesbeiana* were obtained from suppliers in California. The frogs were kept at 17°C on a 12-h light-dark cycle. Two different preparations were studied. In one set of experiments the isolated pigment epithelium-choroid was used; the procedures for using this tissue have previously been described in detail by Miller and Steinberg (1977). In another set of experiments, the retina-pigment epithelium-choroid was used; the procedure for this preparation is similar, and will now be described.

Before an experiment, a frog was light-adapted for several hours under normal fluorescent room lighting. This light adaptation helped to keep the retina attached to the pigment epithelium during the dissection. The frog was then decapitated, and an eye was enucleated and sectioned into anterior and posterior portions. The posterior portion was cut through the optic disc into two sections. One of these sections was submerged in oxygenated Ringer's solution and trimmed into a 6-mm square piece. The sclera was then dissected free from the choroid and discarded. The remaining tissue, consisting of retina, pigment epithelium, and choroid, was mounted on a chip
by the method described by Miller and Steinberg (1977). This chip was placed in the
slot of a perfusion chamber at a 45° angle (Fig. 1).

In this perfusion chamber, each side of the tissue was immersed in a separate 2.8 ml
bath. The baths were named with respect to the side of the pigment epithelium they
faced. The bath on the retinal side of the tissue was termed the apical bath, and the
bath on the choroidal side of the tissue was termed the basal bath. The method of
mounting the tissue as a membrane resulted in electrical isolation of these two fluid

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**Figure 1.** Diagram of the perfusion chamber and the chip on which the tissue
was mounted.

baths. Complete isolation, however, was prevented by the imperfect nature of the seal
(Miller and Steinberg, 1977). The baths were continuously perfused with solutions by
gravity feed from large reservoirs. The rate of flow into each bath was adjusted by
micrometer capillary valves (Roger Gilmont Instruments, Inc., Great Neck, N. Y.) to
5.0 ml/min. The solutions entering each chamber could be rapidly switched by means
of three-way valves (Iso-latch, General Valve Corp., E. Hanover, N. J.). Fluid turbulence
was minimized by having the solutions enter each bath at its bottom, and exit each
bath via a filter paper wick at its top. Each wick emptied the solution into a small
reservoir, which in turn was emptied by a cloth wick into a large bucket. The wicks
eliminated drips, which would have caused pressure pulsations and instability of
microelectrode penetrations.
Solutions

The tissue was usually perfused with 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. This solution was bubbled with 95% O2-5% CO2 and had a pH of 7.4±0.1. The osmolarity was 227 mosM. In one set of experiments, the potassium concentration was varied by equimolar exchange of sodium chloride and potassium chloride. The K+-specific microelectrodes were calibrated in modified Ringer's solutions in which [K+] + [Na+] was kept constant at 112.0 mM. The solutions were at room temperature (20-25°C).

Electrodes

Conventional micropipette electrodes were made from glass capillary tubing (1.0 mm OD, 0.5 mm ID; Omega Dot, Glass Co. of America) on a microelectrode puller designed by K. T. Brown and D. G. Flaming. The electrodes were filled by injection with 5 M potassium acetate, and stored in a humid chamber for 24 h. The electrodes were then beveled on an optically flat surface embedded with 0.05-μm alumina particles (Brown and Flaming, 1975). The beveled electrodes had DC resistances of 40-100 MΩ.

Potassium-specific microelectrodes were constructed by a new technique. Double-barrel glass capillary tubing (two barrels fused side-by-side, each 1.0 mm OD, 0.5 mm ID, Omega Dot) was pulled into an electrode with a single tip of ~0.5 μm. One barrel was siliconized as follows. A single drop of 2.0% tri-methyl-chlorosilane in CC14 was injected into the lumen. This solution filled the tip by capillary action. The electrode was then heated to 190°C for 5 h. Potassium ion exchanger solution (Corning Glass Works, Science Products Div., Corning, N. Y., no. 477317) was injected into the shank of the siliconized barrel with a 30 G needle. This solution filled the tip by capillary action. The lumen of the barrel above the ion exchanger solution and the lumen of the reference barrel were filled with Ringer's solution. The electrode was beveled on a surface embedded with 0.3-μm alumina particles, while the resistance of the reference barrel was monitored. After beveling, the resistance of the reference barrel ranged from 100 to 200 MΩ. A chlorided silver wire placed in each barrel completed the electrode. This type of K+-specific microelectrode has previously been shown to respond to changes in [K+]0 with a time constant of less than 100 ms (Lux, 1974; Oakley, 1975).

Each electrode was calibrated in solutions of varying [K+]0 before and after an experiment. Electrode drift was usually less than 2 mV during a 4-h experiment. An empirical equation of the form

\[
V_{K^+} = \frac{nRT}{F} \log_e \left( \frac{[K^+] + [Na^+]}{S} \right) + V_0.
\]

where \(V_{K^+}\) is the differential potential between the two barrels, \(n\) is a factor (less than 1.0) which accounts for the nonideal slope of the electrode, \(R\) is the gas constant, \(T\) is the absolute temperature, \(F\) is the Faraday, \(S\) is the selectivity coefficient for \(K^+\) over \(Na^+\), and \(V_0\) is a constant (Walker, 1971; Oakley and Green, 1976), was fit to the electrode calibration data by a least-squares regression analysis.

Recording

The recording system allowed simultaneous measurement of both the voltage across the preparation and either intracellular pigment epithelial membrane potentials or extracellular \([K^+]\). Electrical contact between the fluid in the apical chamber and two beakers containing saturated KCl solutions was made by a pair of Ringer's-agar bridges. A calomel electrode was placed in one KCl solution and a chlorided silver wire in the
other. In a similar manner, electrodes were arranged to make electrical contact with the fluid in the basal chamber. The total potential difference across the tissue \( V_T \) was measured between the two calomel electrodes by a differential amplifier with a gain of 10. This potential was further amplified by a gain of 10, and summed with a variable bucking voltage. In this manner the ERG, which was the light-evoked change in \( V_T \), could be recorded with an overall gain of 100. An isolated current source was used to pass current pulses (1.0-5.0 \( \mu \)A) across the tissue (via the two chlorided silver wire electrodes and the symmetrically placed Ringer's-agar bridges) in order to measure the equivalent resistance of the tissue \( R_T \).

A unity gain preamplifier with an input impedance of \( 10^{13} \) \( \Omega \) was used to measure the potential of the microelectrode in a pigment epithelial cell. The apical membrane potential \( V_{Ap} \), measured differentially between the intracellular microelectrode and the fluid on the retinal side of the pigment epithelium, is different from the basal membrane potential \( V_{Ba} \), measured differentially between the intracellular microelectrode and the fluid on the basal side of the pigment epithelium. The difference \( V_{Ba} - V_{Ap} \) is equal to the transepithelial potential (TEP) (Steinberg and Miller, 1973). Thus it was necessary to measure the potential of the intracellular microelectrode differentially against both the apical and the basal calomel reference electrodes.

In recording the potential of the apical membrane, the neural retina was physically interposed between the intracellular microelectrode and the reference electrode in the apical bath. The potential recorded in this manner was consequently the algebraic sum of two potentials: the apical membrane potential \( V_{Ap} \) and the transretinal potential \( V_R \). In an analogous way, the intracellular potential measured against the basal reference electrode was the sum of the potentials across both the basal membrane and the choroid. Since the resistance of the choroid is very small with respect to the basal membrane and there are no known voltage generators in the choroid (Miller and Steinberg, 1977) it was assumed that there was no potential drop across the choroid and that the potential recorded in this manner was a measure of the basal membrane potential alone \( V_{Ba} \). A schematic diagram of these potentials is given in Fig. 2. A reed relay pulsed at 5 Hz was sometimes used to switch between the two references (Steinberg and Miller, 1973), so that the two potentials, \( V_{Ap} + V_R \) and \( V_{Ba} \), could be recorded "simultaneously" as one signal (Fig. 3 A).

The potential difference between the two barrels of the double-barrel, \( K^+ \)-specific microelectrode \( V_{K^+} \) was measured with a differential amplifier. The input stages of this amplifier were capacity compensated and had input impedances greater than \( 10^{14} \) \( \Omega \) (Fig. 3 B). With the electrode tip in the apical bath and the potential of each barrel referred to the reference electrode in the basal bath, the negative capacity for each barrel of the electrode was adjusted so that both barrels recorded the ERG equally. In this manner, the differential voltage between the two barrels \( V_{K^+} \) was not contaminated by purely electrical signals, such as the ERG.

All of the potentials were monitored on an oscilloscope (Tektronix Inc., Beaverton, Ore.) and on digital millivoltmeters (Weston Instruments, Newark, N. J.). The signals were recorded directly on strip chart recorders (Brush 220), as well as on an FM tape recorder (Vetter C4, A. R. Vetter Co., Rebersburg, Pa.). The FM recordings were sampled and digitized by a computer (Nova 2, Data General Corp., Southboro, Mass.), and then plotted (Zeta plotter, model 100, Zeta Research Inc., Lafayette, Calif.).

**Photostimulator**

A single-channel photostimulator provided uniform stimulation of the retina in these experiments. The light source was a 100 W tungsten halogen lamp run at 8.0 A from a constant current power supply. The light beam was focused by aspheric condensing
lenses, collimated, and passed through a water heat filter. The intensity of the light beam was attenuated by a 2.0 log unit circular linear wedge, discrete neutral density filters, and an interference filter with a peak transmission at 500 nm and a 6-nm half-bandwidth (Optical Industries). An electromagnetic shutter (Vincent Associates, Rochester, N. Y.) was placed in the beam, and controlled by a shutter driver unit providing

![Equivalent Circuit](image)

**Figure 2.** Equivalent circuit for recording microelectrode potentials. The resistors represent the resistances of the retina, the choroid, the apical and basal membranes of the pigment epithelium, and two shunt pathways. One shunt resistor represents the edge damage pathway between the apical and basal chambers due to damaged tissue at the edge of the chip. The other shunt resistor represents the pigment epithelial paracellular shunt pathway. The batteries represent the voltages generated across the retina and the apical and basal membranes. The potentials are all described in the text, and are labeled with the polarity with which they were recorded. In all figures the potentials are displayed with positive polarity upwards.

stimulus durations from 5 ms to 50 s. The light beam was reflected off a front surface mirror onto the retina-pigment epithelium-choroid preparation. The unattenuated light beam had a quantum flux of $7.3 \times 10^{13}$ quanta s$^{-1}$ cm$^{-2}$ (at 500 nm), as measured by a calibrated photodiode (United Detector Technology Inc., Santa Monica, Calif.).

**RESULTS**

*The Light-Evoked Pigment Epithelial Cell Response*

In order to test the hypothesis that the c-wave is a manifestation of the response of the pigment epithelium to a light-evoked decrease in $[K^+]_o$, it was first necessary to characterize the light-evoked response of the pigment epithelium. In Fig. 4, an ERG and an intracellularly recorded pigment epithelial cell
response to light are shown. The ERG contained a slow, vitreal-positive c-wave, which was sustained for the duration of the stimulus. The intracellular response was recorded between the intracellular microelectrode and both the apical and basal reference electrodes (the references were switched at 5 Hz).

When measured against the basal reference, the intracellular response was a slow hyperpolarization with the same time course as the c-wave. When measured against the apical reference, the intracellular response was the algebraic sum of the changes in two potentials: the apical membrane potential $V_{Ap}$ and the trans-retinal potential $V_R$. The combined ($V_{Ap} + V_R$) response to light was primarily a slow, negative-going potential, which also had the same time course as the c-wave.
Fast potentials were also recorded in the combined \((V_{AP} + V_R)\) response, as can be seen in the lower half of Fig. 4. It is likely that these potentials were the ERG b- and d-waves, and thus had their voltage sources in the retina. It is unlikely that these potentials were due to actual voltage drops of the b- and d-wave currents across the apical membrane since these currents would have produced depolarizing voltage drops across the apical membrane.

In a preliminary study (Oakley et al., 1977), the slow component of the combined \((V_{AP} + V_R)\) potential was thought to represent primarily a hyperpolarization of the apical membrane, since slow potentials of this polarity and magnitude were not seen before the penetration of the cell membrane. Nevertheless, because of the physical placement of the two recording electrodes, the response attributed to the apical membrane could be due in part to a slow retinal potential with the same time course as the apical hyperpolarization. This slow retinal potential could either add to or subtract from the membrane potential, thus preventing direct measurement of the true apical hyperpolarization.

It was possible, however, to synthesize the response of the apical membrane in an indirect manner, as follows. The light-evoked changes in intracellular potentials were recorded sequentially with respect to each reference electrode. These potentials, \((V_{AP} + V_R)\) and \(V_{BA}\), are shown in Fig. 5. The microelectrode was then withdrawn across the apical membrane 10 \(\mu\)m into the retina. Light-evoked responses were again measured with respect to each reference electrode. The response measured with respect to the apical reference was the potential across the retina, \(V_R\), and the response measured with respect to the basal reference was the potential across the pigment epithelium, or the transepithelial
potential, TEP. These responses are also shown in Fig. 5. The potential across the apical membrane alone, $V_{AP}$, was synthesized (with the computer) by subtracting $V_R$ from $(V_{AP} + V_R)$. The synthesized waveform $V_{AP}$ was not contaminated by any potentials of retinal origin and was a true measure of the light response of the apical membrane. $V_{AP}$ consisted solely of a hyperpolarization of the membrane; the fast ERG components seen in $(V_{AP} + V_R)$ were absent. $V_R$ contained a slow potential of time course similar and polarity opposite to the slow apical hyperpolarization. Thus the combined $(V_{AP} + V_R)$
potential change was smaller in amplitude than the apical hyperpolarization. By knowing $V_{Ap}$ and $V_{Ba}$, it was possible to synthesize the light-evoked change in TEP by subtracting $V_{Ap}$ from $V_{Ba}$. The synthesized waveform $TEP^*$ is also shown in Fig. 5 and is nearly identical to the waveform measured directly.

A light-evoked hyperpolarization of the apical membrane is the type of response that would result from a decrease in $[K^+]_0$ at the apical membrane. However, the responses in Figs. 4 and 5 show that there was a light-evoked hyperpolarization of the basal membrane as well. A previous analysis of pigment epithelial membrane potentials in the isolated pigment epithelium-choroid preparation (Miller and Steinberg, 1977) showed that changes in the apical membrane potential (due to ion concentration changes in the apical bath) were electrically shunted to the basal membrane. The responses in Figs. 4 and 5 are consistent with this previous result in that a light-evoked hyperpolarization of the apical membrane was shunted to the basal membrane, causing it to hyperpolarize as well.

Responses to other stimulus intensities were also measured (not shown) in the same cell from which the responses in Fig. 5 were obtained. In the same manner, the light-evoked changes in $V_{Ap}$ were calculated. From these data several relationships were obtained, as plotted in Fig. 6. As the stimulus intensity was varied, the change in TEP was a linear function of the change in $V_{Ap}$. This was most probably due to shunting of a constant fraction of the apical membrane hyperpolarization to the basal membrane. Like the change in TEP, the c-wave amplitude was a linear function of the apical membrane hyperpolarization. The relationship was somewhat different, however, so that the change in TEP was 1.2 times greater than the c-wave. The change in TEP was greater than the c-wave because the slow component in $V_R$ was of polarity opposite to the change in TEP, and subtracted from it in the recording of the c-wave. Finally, there was a linear relationship between $V_R$ and $V_{Ap}$, meaning that both the apical membrane potential and the slow retinal potential were responding to light stimuli with the same functional relationship.

**Membrane Conductance during the Light-Evoked Hyperpolarization**

In order to rule out the possibility that changes in the concentrations of other ions or molecules could produce the apical hyperpolarization by altering the membrane conductance to potassium and/or bicarbonate, relative membrane conductance was assessed during the hyperpolarization. This was done as follows. Constant current pulses ($I = 5 \, \mu A$) were passed across the tissue in the choroid-to-retina direction. This current produced a voltage drop across the tissue, $\Delta V_T$, as shown in the upper half of Fig. 7. The equivalent resistance of the tissue, $R_T$, was calculated as $R_T = \Delta V_T/I$ ($R_T$ represents the resistance of the equivalent circuit in Fig. 2). Part of the current that flowed across the tissue flowed directly across the pigment epithelial apical and basal membranes. Since the current flowed inward across the basal membrane and outward across the apical membrane, it produced a hyperpolarization of the basal membrane and a depolarization of the apical membrane, as shown in the lower half of Fig. 7. The current also produced a voltage drop across the retina, $\Delta V_R$, although the voltage drop across the retina was found to be less
than 5% of the voltage drop across the apical membrane, \( \Delta V_{AP} \). Since \( \Delta V_R \) was much less than \( \Delta V_{AP} \), \( \Delta V_{AP} \) was approximately equal to \( \Delta(V_{AP} + V_R) \). The ratio of the absolute magnitudes of the apical-to-basal voltage drops is termed \( a \), and is a measure of the relative apical-to-basal membrane resistances (Frömter, 1972; Miller and Steinberg, 1977).

The value of \( a \) was measured in a repetitive manner so that any changes in membrane conductance could be measured before, during, and after a light stimulus. In order to see if the current pulses themselves had any effect on the tissue, a control experiment was performed in which only a long train of pulses was given. The responses are shown in Fig. 8 A. The train of current pulses did not affect \( R_T \) or \( a \). A light stimulus was then given during a similar series of pulses. The responses are shown in Fig. 8 B. Although this stimulus caused an 8-mV hyperpolarization of the apical membrane, there were no changes in \( R_T \) or \( a \) during the hyperpolarization. Thus there did not appear to be any changes in relative membrane conductance associated with the membrane hyperpolarization.

**Figure 6.** The c-wave, \( \Delta TEP \), and \( \Delta V_R \) as a function of the calculated apical membrane hyperpolarization \( \Delta V_{AP} \). The data were obtained from the same cell as in Fig. 5 for various stimulus intensities. \( \Delta V_{AP} \) was measured from the synthesized waveforms. The solid lines are linear least-squares regression lines.
Measurement of \([K^+]_0\) during Light Stimulation

The light-evoked hyperpolarization of the apical membrane of the pigment epithelium is most likely to be caused by a light-evoked decrease in \([K^+]_0\) in the distal retina (Oakley and Green, 1976). Membrane hyperpolarization increases the TEP (Fig. 5). If the same relationship between the changes in TEP and \([K^+]_0\) is found both when \([K^+]_0\) is altered by light stimulation and when \([K^+]_0\) is altered by another means (direct perfusion of the apical membrane of the isolated pigment epithelium-choroid preparation), then it can be concluded that the change in \([K^+]_0\) alone, by its effect on the apical membrane potential,

\[
V_T
\]

\[
5 \mu A
\]

\[
20 \text{ mV}
\]

\[
V_{BA}
\]

\[
10 \text{ mV}
\]

\[
V_A
\]

FIGURE 7. Measurement of relative membrane conductances (see Materials and Methods). The voltage drops produced by two 5-\(\mu\)A current pulses (horizontal bars) are shown. The upper curve shows the voltage drop across the entire tissue. The lower curve shows the voltage drops across the basal and apical membranes. Between the two current pulses, the microelectrode reference was switched so that the first half of the curve shows the hyperpolarization of the basal membrane by one current pulse, and the second half shows the depolarization of the apical membrane by the other current pulse. The fast voltage transients seen in \(V_{AP}\) at the onset and offset of the current pulse are stimulus artifacts. The ratio of the apical voltage drop to the basal voltage drop \(a\) is a measure of the relative membrane conductances. In this case, \(a\) equals 0.47.

is sufficient to cause the change in TEP. If \([K^+]_0\) causes the apical membrane hyperpolarization by altering the K+ equilibrium potential across the membrane (and there are no voltage-dependent conductance changes or changes in the rate of an electrogenic pump), then there should be a linear relationship between the amplitude of the hyperpolarization (\(\Delta V_{AP}\)) and the logarithm of \([K^+]_0\). Since there is a linear relationship between \(\Delta V_{AP}\) and the change in TEP (\(\Delta TEP\)), there should also be a log-linear relationship between \(\Delta TEP\) and \([K^+]_0\). In order to test these hypotheses, the light-evoked changes in TEP and \([K^+]_0\) were quantified.

A K+ -specific microelectrode was advanced in 5-\(\mu\)m steps through the retina. The steady value of \(V_{K^+}\) increased as a function of the depth of penetration, indicating that the concentration of K+ was higher in the distal retina than in the proximal retina. After the electrode had been advanced \(>200 \mu\)m into the retina from the vitreal border, a large, negative DC shift in the potential of the reference barrel was observed as the electrode penetrated a pigment
epithelial cell. The electrode was then withdrawn ~10 μm into the neural retina. At this retinal depth, the light-evoked changes in both $V_{K^+}$ and $V_T$ (which is the ERG) were measured for various stimulus intensities, as shown in Fig. 9. Both the c-wave and the change in $V_{K^+}$ increased in amplitude as the stimulus intensity was increased. The most intense stimulus (~1 log unit) evoked a c-wave of maximum amplitude.

![Figure 8](image)

**Figure 8.** Measurement of relative membrane conductance. The response were recorded as in Fig. 7 and are displayed on a compressed time scale. A (control), A long train of current pulses was given, and it had no effect on the calculated values of $R_T$ or $a$. B (during a light stimulus), The preparation was stimulated by a light flash (horizontal bar) of ~2 log unit intensity. The value of $a$ at the peak of the light response (0.45) did not differ from the value of $a$ before (0.46, 0.45) or after (0.45, 0.45) the flash.

The value of $V_{K^+}$ was 6 mV higher in the retina, just proximal to the apical membrane, than it was in the retinal perfusion solution which contained 2.0 mM $[K^+]_o$. On the basis of the electrode calibration curve in Fig. 10, $[K^+]_o$ at the apical membrane was 3.1 mM. For each stimulus intensity, the value of $V_{K^+}$ at the peak of the c-wave was plotted on the calibration curve in Fig. 10; the most intense stimulus (~1 log unit) decreased $[K^+]_o$ from 3.1 mM to 1.0 mM. For each of the points in Fig. 10, a corresponding c-wave amplitude was measured from the data in Fig. 9. When the two sets of data were combined,
FIGURE 9. ERGs and $V_{K^+}$ changes in response to stimuli of varying intensity. The light stimulus (horizontal bar) was increased in intensity in 1-log unit steps from $-7$ to $-1$ log unit. The $K^+$-specific microelectrode tip was positioned near the retinal side of the apical membrane of the pigment epithelium.

FIGURE 10. $[K^+]_o$ at the peak of the c-wave. The solid line is a least-squares fit of Eq. (1) to the electrode calibration data. Outside the apical membrane, $V_{K^+}$ was 6 mV higher than it was in the apical bath ($\bullet$), indicating a $[K^+]_o$ of 3.1 mM. For each stimulus intensity, the measured change in $V_{K^+}$ at the peak of the c-wave (Fig. 9) is plotted on the electrode calibration curve (O). The stimulus intensity is labeled next to each point. The maximum intensity stimulus decreased $V_{K^+}$ by 13 mV and correspondingly lowered $[K^+]_o$ from 3.1 to 1.0 mM.
The c-wave amplitude was plotted in Fig. 11 (open circles) as a function of [K+]o at the peak of the c-wave. The data are best represented by a log-linear curve with a slope of −7.9 mV per decade change in [K+]o.

In Fig. 6, it was shown that the change in TEP was 1.2 times as large as the c-wave. Multiplying by 1.2 each of the c-wave voltages in Fig. 11 yields the data represented by the filled squares, which are the calculated changes in TEP during the c-wave. The line through the filled squares has a slope of −9.5 mV (−7.9 × 1.2) per decade change in [K+]o.

**Figure 11.** The amplitudes of the c-wave and ΔTEP as a function of [K+]o at the peak of the c-wave. The c-wave data were obtained from Figs. 9 and 10. The ΔTEP data were calculated by multiplying each of the c-wave amplitudes by 1.2 (see text). The abscissa has a logarithmic scale, and the solid lines through the data points are log-linear least-squares regression lines having slopes of −7.9 mV (c-wave) and −9.5 mV (ΔTEP) per decade change in [K+]o.

The K⁺ Sensitivity of the Transepithelial Potential

The data in the previous section do not rule out the notion that the hyperpolarization of the apical membrane, and thus the increase in TEP, are produced in part by simultaneous changes in [K+]o and in the concentrations of other ions, such as bicarbonate. However, if there were no changes in any ion but K⁺, then the slope of the line through the filled squares in Fig. 11 would represent the sensitivity of the TEP to K⁺ alone.

It was possible to measure directly the effect of apical [K+]o on the TEP. This experiment was performed on the isolated pigment epithelium-choroid preparation (Steinberg and Miller, 1973; Miller and Steinberg, 1977). Changes in TEP were measured as [K+]o was varied in the apical perfusion solution.
Although Miller and Steinberg (1977) performed the same experiment in the range of 0.2-55 mM $[K^+]_o$, the experiment was repeated to obtain more data points in the region of interest (1.0-4.0 mM $[K^+]_o$).

In Fig. 12, changes in TEP, $V_{AP}$, and $V_{BA}$ are shown in response to a 4.0 mM to 1.0 mM change in apical $[K^+]_o$. This decrease in $[K^+]_o$ produced an effect very similar to that of light in the retina-pigment epithelium-choroid preparation (Figs. 4 and 5); that is, a hyperpolarization of $V_{AP}$ and $V_{BA}$ and an increase in TEP. However, these responses were generated more slowly than the light-evoked responses, since the change in $[K^+]_o$ had a slower time course with the perfusion system than it did when changed directly by the retina.

The change in TEP was quantified in several experiments for changes in $[K^+]_o$ in the apical bath. The means of these data are plotted in Fig. 13, along with data previously obtained from this experiment by Miller and Steinberg (1977). The points are best represented by a log-linear curve with a slope of $-9.2$ mV per decade change in $[K^+]_o$. This is essentially the same result found when $[K^+]_o$ was altered during a flash of light. The change in TEP in the retina preparation, therefore, must have been produced solely by $[K^+]_o$.

**DISCUSSION**

**The Origin of the c-Wave**

The method used to record the apical membrane potential (Fig. 4) resulted in contamination of the membrane potential with retinal potentials. Most apparent were the fast retinal potentials (b- and d-waves) which added directly to the membrane hyperpolarization. This type of contamination was seen previously
in pigment epithelial cell recordings in the cat, where a prominent b-wave was superimposed upon the apical membrane response (Steinberg et al. 1970). The method used to synthesize the apical membrane potential (Fig. 5), however, revealed the presence of a slow retinal potential having the same time course as the apical hyperpolarization. This slow retinal potential was vitreal negative,

**Figure 13.** Change in TEP as a function of \([K^+]_0\) in the apical bath. In the isolated pigment epithelium preparation, the changes in TEP were quantified as \([K^+]_0\) in the apical bath was varied. The open circles (○) represent the changes in TEP found in the present experiments when \([K^+]_0]\) was changed from 4.0 mM to either 1.0, 2.0, or 3.0 mM. Each point is the mean value obtained from two such changes in each of three tissues. The filled circles (●) represent the data of Miller and Steinberg (1977) from a similar experiment. The data have been shifted vertically, so that ∆TEP equals 0.0 when \([K^+]_0]\) equals 4.0 mM. The abscissa has a logarithmic scale, and the solid line is a log-linear least-squares regression line (representing the open circles) having a slope of −9.2 mV per decade change in \([K^+]_0\).
so it subtracted from the change in TEP, making the ERG c-wave (recorded across the entire preparation) smaller than the change in TEP. Since the retinal potential was much smaller than the change in TEP, to a first approximation, the ERG c-wave represented the light-evoked increase in TEP. The slow retinal potential could be due to a voltage source in the retina and/or to current flow from a remote voltage source. For example, the apical membrane hyperpolarization would cause current to flow in the pigment epithelial → vitreal direction (see Fig. 2), and would thus cause a vitreal-negative potential drop across the retina.

Faber (1969) showed that in the rabbit the ERG c-wave was the sum of a pigment epithelial and a retinal component. When the pigment epithelial component was eliminated by transretinal current, a corneal-negative slow potential was recorded, which Faber called slow PIII. On the basis of the similarity of the stimulus-response characteristics of slow PIII and the c-wave, Faber suggested that the pigment epithelial component of the c-wave and slow PIII were related to the same basic process in vision. Witkovsky et al. (1975) suggested that slow PIII is generated by the response of the Müller cells to a decrease in \([K^+]_0\). These ideas were supported in the present study by the finding that the amplitude of the slow retinal potential \(V_R\) was a linear function of the change in TEP, which in turn was a log-linear function of the change in \([K^+]_0\).

Rodieck (1972) also found that the c-wave was the sum of two components: the "late receptor component" and the "r-component." The late receptor component was thought to originate in the retina, whereas the r-component was thought to originate in the pigment epithelium. Rodieck's analysis of the cat c-wave showed that the retinal and pigment epithelial components were about equal in amplitude but opposite in polarity. The present experiments show that in the frog, the pigment epithelial component is much larger than the retinal component (Figs. 5 and 6). The differences are likely to be due to species variability. It is unlikely that in the present experiments the amplitude of the retinal component was altered by the presence of the intraretinal microelectrode. The c-wave, which is the sum of the pigment epithelial and retinal components, did not change in amplitude when the retina was penetrated by the microelectrode. In addition, Rodieck (1972) used an analogous microelectrode technique to measure the large r-component in the cat.

The Shunt

A decrease in \([K^+]_0\) outside the apical membrane caused a membrane hyperpolarization, whether this decrease in \([K^+]_0\) resulted naturally from a flash of light or was produced artificially in the solution perfusing the isolated pigment epithelium. Even though the ion concentration changes took place outside the apical membrane, the basal membrane hyperpolarized with a similar, slow time course. Miller and Steinberg (1977) showed that such a response is due to a shunt pathway in vitro, so that voltage changes produced by one membrane are electrically shunted to the other. The shunt also reduces the measured amplitude of the apical hyperpolarization, due to the voltage drop produced by the current flow through the apical membrane resistance (see Fig. 2). If
not for the shunt, the change in TEP in this preparation would be much larger in amplitude; it could be as large as the change in $V_{AP}$.

The shunt pathway appears to be purely resistive, so that a constant fraction of the change in $V_{AP}$ is shunted to the basal membrane (Fig. 6). As a result of this linearity, the change in TEP varies linearly with the hyperpolarization of the apical membrane.

The shunt is most probably composed of two separate pathways. One shunt pathway is a paracellular one due to the intercellular junctional complexes (Hudspeth and Yee, 1973). The other arises from damaged cells at the edge of the tissue due to the nature of the mechanical seal (Miller and Steinberg, 1977). The paracellular pathway would be the same in vivo, whereas the edge-damage shunt would be replaced by a shunt pathway involving other tissues in the eye (Rodieck, 1973). These two shunt pathways act as resistors in parallel, and are likely to have a significant effect in vivo. If these shunt resistances were much larger in vivo, then the amplitude of the c-wave would be larger in vivo than it is in vitro. Lurie (1976) recorded c-waves from the intact frog eye, and they were no larger than those recorded in vitro in the present experiments. In addition, the data of Steinberg et al. (1970) showed that the magnitude of the light-evoked potential across the apical membrane and the retina in vivo was ≈3 times larger than the c-wave. This difference is probably due to the shunt.

The $[K^+]_o$ Response

In the distal retina, $[K^+]_o$ was 3.1 mM, compared with 2.0 mM in the solution perfusing the vitreal surface of the retina. Such a difference has been reported previously by Oakley and Green (1976) who found that $[K^+]_o$ ranged from >5 mM in the distal retina to 2.5 mM in the vitreous. The lower level of $[K^+]_o$ in the distal retina found in the present study could be due to the steady perfusion of the vitreal side of the tissue with 2.0 mM $[K^+]_o$ solution, since the higher level of $[K^+]_o$ was found in the frog eyecup preparation (not perfused).

The light-evoked hyperpolarization of the apical membrane appears to result solely from a decrease in $[K^+]_o$ at the apical membrane. It is likely that the change in $[K^+]_o$ has its effect directly on the $K^+$ equilibrium potential of the apical membrane. From the data in this paper, it is not possible to rule out effects of $[K^+]_o$ on an electrogenic pump in the apical membrane. However, Miller and Steinberg (1977) found that the response of the apical membrane to $[K^+]_o$ is unchanged 10 min after the application of $10^{-4}$ M ouabain to the apical membrane, which was sufficient to block pump activity. Therefore, the effect of the light-evoked decrease in $[K^+]_o$ is most likely a direct effect on the $K^+$ equilibrium potential.

A log-linear relationship was found to exist between the peak value of the c-wave and $[K^+]_o$. The basis for this relationship was found to be the $K^+$ electrode behavior of the pigment epithelial apical membrane. Since the decrease in $[K^+]_o$ in the distal retina occurs on a very slow time scale, it is likely that the apical membrane potential (and thus the c-wave) is a measure of the instantaneous level of $[K^+]_o$. Thus, a certain c-wave voltage should be produced whenever $[K^+]_o$ decreases from its base-line level by a fixed amount,
no matter when during the response that change occurs. This prediction was verified as follows.

In Fig. 14, two of the light-evoked responses from Fig. 9 are repeated. When \( V_{K^+} \) (and thus \( [K^+]_0 \)) fell to the level represented by the filled circles, the instantaneous c-wave voltage produced was the same, independent of stimulus intensity. This relationship was also found between the changes in \( V_{K^+} \) and the c-wave voltages represented by the open squares. However, this relationship broke down at early times after the onset of stimulus, as shown by the plus symbols. This result is not surprising since at this time the ERG contained both a c-wave and a decaying b-wave (the relationship also broke down after the stimulus offset, due to the d-wave). Thus, the c-wave voltage is a measure of the instantaneous, light-evoked decrease in \( [K^+]_0 \) in the distal retina.

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