Effects of Current Clamp on Chick
Retinal Pigment Epithelium

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The basal membrane of the retinal pigment epithelium (RPE) is the origin of two components of the electroretinogram, the fast oscillation and the light peak. Both of these responses originate from changes in basal membrane potential \( V_{\text{ba}} \), and both are associated with changes in basal membrane resistance \( R_{\text{ba}} \). In addition, many experimental manipulations that alter \( V_{\text{ba}} \) also produce apparent changes in \( R_{\text{ba}} \). These findings raise the possibility that the basal membrane contains a voltage-sensitive conductance that operates in the physiologic range and is involved causally in light-evoked and other responses. We report the results of current clamp experiments on the isolated retina-RPE-choroid of chick that were designed to test for the presence of such a voltage-sensitive conductance in the basal membrane. Depolarizing \( V_{\text{ba}} \) by 15 mV with retina-to-choroid current had essentially no effect on either the ratio of membrane resistances \( \frac{R_{\text{ap}}}{R_{\text{ba}}} \) or the transistissue resistance \( R_{\text{tot}} \), indicating no alteration in \( R_{\text{ba}} \). In contrast, hyperpolarizing \( V_{\text{ba}} \) by 15 mV with choroid-to-retina current caused a gradual decrease in \( R_{\text{tot}} \) and increase in \( \frac{R_{\text{ap}}}{R_{\text{ba}}} \). Analysis of accompanying changes in membrane voltages and changes in intracellular c-wave amplitude suggested that the most likely cause of the decrease in \( R_{\text{tot}} \) is a decrease in paracellular resistance. Voltage-sensitive conductances of the basal membrane appear to play little or no role in the resistance changes that accompany changes in \( V_{\text{ba}} \) in the physiologic range. The conductance changes underlying the fast oscillation and light peak probably result from either the modulation of channels by second messengers or changes in intracellular ion concentration.

Illumination of the dark-adapted retina evokes a series of voltage changes that can be recorded noninvasively in the direct-current electroretinogram (DC ERG). Three components of the DC ERG originate from the retinal pigment epithelium (RPE): the c-wave, the fast oscillation, and the light peak. The c-wave component arises from the RPE apical membrane as a voltage response to a light-evoked decrease in subretinal K⁺ concentration and is mediated by a Ba²⁺-inhibitable K⁺ conductance. The fast oscillation, which follows the c-wave, originates from a delayed hyperpolarization of the basal membrane, and this (in turn) is followed by the light peak, which originates as a much slower depolarization of the same membrane. A striking aspect of these two basal membrane voltage responses is that they are associated with changes in basal membrane apparent resistance.

It increases as the membrane hyperpolarizes during the fast oscillation, and it decreases as the membrane depolarizes during the light peak.

It is not known how these resistance changes are involved in the mechanisms at the basal membrane that generate these two responses. Several mechanisms could explain these parallel voltage and resistance changes: (1) increases or decreases in the conductance for an ion whose equilibrium potential is positive to the resting potential; (2) changes in the intracellular activity of a permeant ion, leading to changes in both equilibrium potential and conductance for the ion; (3) changes in electrogenic pump activity, with secondary effects on a voltage-sensitive conductance that activates with membrane depolarization and deactivates with hyperpolarization; or (4) a combination of mechanisms 1, 2, and 3.

We investigated whether the basal membrane possesses voltage-sensitive conductances that might contribute to either/or both the fast oscillation and the light peak by current clamping the isolated chick retina–RPE–choroid. This preparation exhibits both a fast oscillation and light peak and has a permeable choroid that allows changes in solution composition outside the basal membrane. Our results show that in the voltage range over which the fast oscillation and light peak occur, there is no evidence for a basal mem-
brane conductance that is either activated by depolarization or deactivated by hyperpolarization.

Materials and Methods

Dissection

The retina–RPE–choroid was isolated from chick eyes as previously described. Briefly, white chicks (Gallus domesticus), 1–14-days-old, were light adapted for at least 2 hr to promote retinal adhesion and then were dark adapted for 10 min before decapitation to reduce the possibility of spreading depression. All animals were maintained and cared for in accordance with the ARVO Resolution on the Use of Animals in Research. Each eye was enucleated and mounted, cornea-side down, in a dissection chamber filled with control perfusate. A circular section of retina–RPE–choroid, approximately 4–8 mm in diameter, was dissected through an incision in the sclera and placed in a recording chamber that allowed the two surfaces of the preparation to be perfused independently. The solutions were delivered to the chamber by gravity feed through CO2 impermeable tubing (Saran; Clarkson, Detroit, MI) and removed by suction. An open manifold (~250 µl) separated the solution reservoirs, and the chamber and provided a constant hydrostatic head. The solutions were maintained at 37°C by water jacketing the solution reservoirs and by flowing the solutions over a heat exchanger just before they entered the chambers.

Solutions

Chick Ringer’s solution consisted of: 120.0 mM NaCl, 25.0 mM NaHCO3, 25.0 mM glucose, 5.0 mM KCl, 3.0 mM MgCl2, and 1.8 mM CaCl2. This solution was bubbled constantly with 95% O2 and 5% CO2 at a temperature of 37 ± 1.0°C and had a pH of 7.4 ± 0.1. In some experiments, BaCl2 or 4,4'-diisothiocyanostilbene-2,2'-disulfonate (DIDS) were added to the basal perfusate. All chemicals were purchased from Sigma (St. Louis, MO).

Stimuli

A halogen lamp delivered a diffuse white light stimulus through a mirror for a final stimulus intensity of 1.8 × 10^-3 W/cm². C-waves were elicited by 4-sec flashes at intervals of 60 sec. Before recording, the tissue was dark adapted for approximately 1 hr.

Electrophysiology

Conventional microelectrodes were drawn from 1.0-mm glass tubing (Omega Dot; Glass Company of America, Millville, NJ) with a horizontal puller (model P-77; Sutter, San Francisco, CA), filled with 150 mM KCl and then beveled to a resistance of 100–150 MΩ. Techniques for electrophysiologic recordings were similar to those used previously. In both the retinal and choroidal compartments of the recording chamber, a calomel electrode made electrical contact with the perfusion solution through a Ringer’s-agar bridge. Current was passed across the tissue between a chlorided Ag wire ring in the retinal bath and a Ag/AgCl pellet in the choroidal bath. Positive current was in the retina-to-choroid direction. Micro-electrodes were placed either in the subretinal space or in the RPE cell. All voltage signals were digitized at a rate of 4 or 5 Hz and stored on a microcomputer for subsequent analysis.

Equivalent Electrical Circuit

The equivalent circuit of the retina–RPE–choroid is shown in Figure 1. Placed in the subretinal space (position 2), the microelectrode provided the transepithelial potential (TEP) when referenced to the choroidal electrode (position 5) and the transepithelial potential (V) when referenced to the retinal electrode (position 1). The intracellular recording (position 3) gave the basal membrane potential (Vba) when referenced to the choroidal electrode. When referenced to the retinal electrode, it gave the sum of apical membrane potential (Vap) and Vr, which we term Vapp.

The transepithelial potential (Vch) and the apparent ratio of the apical to basal membrane resistance (Rap/Rba) are given by:

\[
\frac{V_{app}}{V_{ba}} = \frac{R_{ap}}{R_{ba}}
\]

where

\[
V_{ch} = V_{app} + V_{ba} = \frac{V_{ba}}{R_{ba}}\frac{R_{ch}}{R_{ch} + R_{ba}} V_{r}
\]

and

\[
V_{r} = V_{ba} + V_{ap}
\]

Fig. 1. Equivalent circuit of the neural retina and RPE-choroid (ref. 7; reproduced with permission of publisher). The neural retina is represented by a resistor RR and battery VR. The apical and basal membranes of the RPE are represented by batteries Vap and Vba. RR represents the combined resistance of the paracellular and "tissue-edge" pathways. The transepithelial potential (TEP) is the potential across the RPE and the transepithelial potential (TTP) is the sum of TEP and VR.
were obtained by passing DC current pulses across the tissue and monitoring the voltage deflections across the entire tissue and across the apical and basal membranes. For these calculations, we used the difference between voltages recorded just before and between 200 and 300 msec after the change in current clamp. The \( R_{ap}/R_{ba} \) calculated from measurements in the retina–RPE–choroid preparation was not corrected for voltage drops across the retinal resistance, and thus it somewhat overestimates the true resistance ratio. Current pulses could be presented by themselves or superimposed on constant current.

In the equivalent circuit of Figure 1, the apical membrane is modeled as a resistance (\( R_{ap} \)) in series with a battery, \( (V'_{ap}) \). Likewise, the basal membrane is modeled as a resistance (\( R_{ba} \)) in series with a battery (\( V'_{ba} \)). These membrane resistances are shunted by a resistor (\( R_s \)) which is the combined paracellular and “tissue-edge” pathways. Equations for open circuit TEP, \( V_{ap} \) and \( V_{ba} \) have been derived previously. According to the equivalent circuit in Figure 1, the equation relating these resistive elements to the trans-epithelial resistance (\( R_T \)) is:

\[
R_T = \frac{R_s(R_{ap} + R_{ba})}{R_{ap} + R_{ba} + R_s}
\]

Equation 1 indicates that \( R_T \) can decrease from a decrease in either of the membrane resistances or the shunt resistance.

**Results**

**Effects of Brief Current Pulses on the Retina–RPE–Choroid**

To test for the presence of a voltage-sensitive conductance in the RPE basal membrane, we measured the membrane voltage responses to brief current pulses of opposite polarity. If a rapidly activating conductance were present, then we would expect the basal membrane response to a hyperpolarizing pulse to differ in magnitude from the response to a depolarizing pulse. Figure 2A shows the voltage response of the trans-tissue potential (TTP), \( V_{ba} \), and \( V_{ap} \) \( (V_{ap} \) and \( V_{ba} \) in series) to 3-sec current pulses passed across the tissue. In this and most other experiments, bipolar current pulses were applied to avoid a cumulative effect of solute polarization. The amplitude of these current pulses, \( \pm 10 \mu A \), changed \( V_{ba} \) by \( \pm 8 \) mV, which encompasses the values of \( V_{ba} \) attained during the fast oscillation and light peak of the chick ERG. The upper panel of Figure 2A shows that current pulses in either direction produced biphasic changes in TTP; an initial voltage deflection at the onset of current was followed by a small, time-dependent secondary increase in voltage. Similar biphasic voltage changes occurred at the apical membrane, with \( V_{ap} \) also undergoing a secondary voltage increase after an initial step voltage change (lower panel). This pattern of voltage changes was seen in six tissues. In contrast, step voltage changes in \( V_{ba} \) were followed by very small repolarizations toward baseline. In two of six tissues, \( V_{ba} \) also increased after the initial voltage deflection.

To examine the possibility that the time-dependent changes in \( V_{ap} \) and \( V_{ba} \) resulted from changes in membrane and/or paracellular resistances, we calculated \( R_{Total} \) and \( R_{ap}/R_{ba} \) from voltage deflections at the beginning and end of the current pulses. Figure 2B plots \( R_{Total} \) (top panel) and \( R_{ap}/R_{ba} \) (bottom panel) calculated from the voltage deflections at the onset of positive (retina-to-choroid) current (open circles), at the transition from positive to negative current (closed circles), and at the end of negative current (open triangles). The \( R_{Total} \) and \( R_{ap}/R_{ba} \) were essentially identical at each of the times, indicating that during these secondary, time-dependent voltage changes, changes in membrane and/or shunt resistances were either small or absent. These results suggest that in the voltage range of \( -45 \) to \( -60 \) mV, the basal membrane behaves as an ohmic resistor and lacks a conductance that is rapidly activated by mild depolarization.*

**Effects of Long-Term Current Clamp on the Retina–RPE–Choroid**

Although the experiments did not show any resistance changes produced by brief changes in membrane voltage, it was still possible that the basal membrane contained a voltage-dependent conductance that activates with a slower time course. To test this possibility, we measured membrane voltages, \( R_{Total} \) and \( R_{ap}/R_{ba} \) while clamping current in either the retina-to-choroid or choroid-to-retina direction for periods of 1 min or more.

**Effects of retina-to-choroid current clamp:** Figure 3A shows the results of a representative experiment in which a retina–RPE–choroid preparation was current clamped by \( \pm 15 \mu A \) for 100 sec, depolarizing \( V_{ba} \) initially by about \( 15 \) mV. Periodic bipolar current pulses, \( \pm 6 \mu A \), were superimposed on the sustained

* Since the small, time-dependent voltage changes in \( V_{ap} \) and \( V_{ba} \) were not associated with any detectable change in resistance ratios, they reflect a change in membrane or shunt batteries. That these membrane potential changes were generally in the same direction but larger at the apical membrane than at the basal membrane suggests that they originated primarily from a change in \( V_{ap} \).

This postulated change in \( V_{ap} \) could be a manifestation of solute polarization caused by the transport number effect, \( T^{+} + e \) or it could be due to current-induced changes in intracellular ion activity.
current for the determination of $R_{ap}/R_{ba}$ and $R_{Total}$. As shown in the top trace in Figure 3A, retina-to-choroid current produced a step increase in TTP that was sustained for the duration of the current clamp.

Similarly, $V_{ba}$ and $V_{ap}^{app}$ underwent voltage deflections at the onset of the current clamp that remained stable until open circuit was restored. Figure 3B plots $R_{Total}$ and $R_{ap}/R_{ba}$ for the voltage deflections evoked by the superimposed current pulses and shows that neither $R_{Total}$ nor $R_{ap}/R_{ba}$ was affected by retina-to-choroid current clamp. Similar results were obtained when the current clamp was maintained for as long as 3.5 min. These results provide further evidence that the basal membrane of the chick RPE lacks a conductance that is activated by moderate depolarization.

Effects of choroid-to-retina current clamp: In contrast to retina-to-choroid current, choroid-to-retina current produced appreciable time-varying changes in both membrane voltage and resistance. Figure 4A shows the results of one experiment in which a retina–RPE–choroid preparation was current clamped by $-15 \mu A$ for 135 sec, transiently hyperpolarizing $V_{ba}$ by about 15 mV. The TTP response to choroid-to-retina current (top trace) was biphasic, reversing polarity at the onset of current clamp and then decaying monotonically. At clamp offset, TTP abruptly went positive to a level less than the pre-clamp baseline and then slowly repolarized.

The two middle traces of Figure 4A show that these time-dependent changes in TTP had contributions from both $V_{ba}$ and $V_{ap}^{app}$. After a step hyperpolarization of 13 mV, $V_{ba}$ slowly depolarized 4.2 mV. The voltage changes in $V_{ap}^{app}$ were more complex; after a step depolarization of 5.3 mV, there was a further depolarization of 1.0 mV (difficult to see with this scale and time base), which was followed by a slower hyperpolarization of 1.5 mV. As these slow changes in voltage occurred in response to sustained current
clamp, the changes in $V_{ba}$ and $V_{ap}^{app}$ produced by the superimposed current pulses became smaller.

Figure 4B shows that choroid-to-retina current changed $R_{Total}$ and $R_{ap}/R_{ba}$ with a time course similar to the slow changes in membrane voltage. The $R_{Total}$ decreased nearly 50% from 1100 to 600 Ω while $R_{ap}/R_{ba}$ nearly doubled from 0.35 to 0.65. Both resistance parameters slowly returned toward their initial values after the return to open circuit, but recovery was incomplete even after 30 min.

Table 1 summarizes the results of similar experiments in eight tissues. Time-varying depolarizations of $V_{ba}$ were observed in every cell and averaged 3.1 ± 0.5 mV (mean ± standard error of the mean). Hyperpolarizations of $V_{ap}^{app}$ were observed in eight of the nine cells and averaged 0.7 ± 0.3 mV; these values may be underestimated because of some temporal overlap with the secondary depolarization that follows the initial voltage deflection (Fig. 2A). Table 1 also shows that choroid-to-retina current decreased $R_{Total}$ by an average of 33% and increased $R_{ap}/R_{ba}$ by an average of 43%.

Previous studies suggested that the RPE basal membrane contains both $K^+$ and $Cl^-$ conductances. To test the possibility that these conductances might mediate the current-induced voltage and resistance changes, we tested the effect of choroid-to-retina current after superfusing the basal side of the tissue with either the $Cl^-$ channel blocker DIDS or the $K^+$ channel blocker Ba$^{2+}$. The responses to brief current were unaffected by basal DIDS (0.5 mM) or basal Ba$^{2+}$ (5 mM) (not shown), suggesting that neither a basal membrane $Cl^-$ channel nor a $K^+$ channel were involved.
Fig. 4. Effect of choroid-to-retina (negative) current clamp on membrane voltage (A) and resistance (B) in a retina-RPE-choroid preparation. Extrinsic current was clamped from 0 to \(-15\) \(\mu\)A for 135 sec, transiently hyperpolarizing \(V_{\text{op}}\) by about 15 mV; superimposed on this steady current were pulses used for the calculation of \(R_{\text{Total}}\) and \(R_{\text{ap}}/R_{\text{ba}}\) in (B). Negative current produced a decline in \(R_{\text{Total}}\) and an increase in \(R_{\text{ap}}/R_{\text{ba}}\). Abbreviations as in Figure 2.

Effect of Current Clamp on the RPE–Choroid

To investigate the possible role of the neural retina in the development of voltage and resistance changes produced by choroid-to-retina current, we examined the effect of current clamp in isolated RPE–choroid preparations. Figure 5A shows that passing \(-45\) \(\mu\)A across an isolated RPE–choroid produced time-dependent changes in TEP, \(V_{\text{ba}}\), and \(V_{\text{ap}}\) that were qualitatively similar to those in the retina–RPE–choroid preparation, but smaller in magnitude, despite the larger step changes in \(V_{\text{ba}}\) and \(V_{\text{ap}}\). Figure 5B plots the time course of changes in transepithelial resistance \(R_{\text{t}}\) and \(R_{\text{ap}}/R_{\text{ba}}\) calculated from the data in Figure

Table 1. Effect of choroid-to-retina current on membrane voltages and resistance ratios (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>(TTP)</th>
<th>(V_{\text{ap}})</th>
<th>(V_{\text{op}})</th>
<th>(R_{\text{ap}}/R_{\text{ba}})</th>
<th>(R_{\text{Total}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>-13.6 ± 1.2</td>
<td>-54.5 ± 1.2</td>
<td>-68.1 ± 1.3</td>
<td>0.37 ± 0.04</td>
<td>1115 ± 55</td>
</tr>
<tr>
<td>t = 2 min</td>
<td>-9.8 ± 1.1</td>
<td>-55.2 ± 1.1</td>
<td>-65.0 ± 1.1</td>
<td>0.53 ± 0.05</td>
<td>742 ± 45</td>
</tr>
<tr>
<td>Time-dependent change</td>
<td>+3.8 ± 1.1</td>
<td>-0.7 ± 0.3</td>
<td>+3.1 ± 0.5</td>
<td>0.16 ± 0.03</td>
<td>-373 ± 84</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
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</table>

Initial voltage values were measured at the peak of the initial \(V_{\text{ba}}\) hyperpolarization. TTP reversal, and the \(V_{\text{ap}}\) depolarization that followed current onset; values were measured again after 2 min of current clamp. Resistance ratios were calculated from voltage responses elicited by 3-sec current pulses before and after 2 min of current clamp. The time-dependent changes in voltage and resistance ratios are the difference between values at \(t = 2\) min and initial values.
Fig. 5. Effect of negative current clamp on membrane voltage and resistance in the isolated RPE-choroid preparation. Current clamp protocol similar to that in Figure 4 except that 45 μA of current was used.

5A and shows that negative current also caused a decrease in R, and an increase in Rb/Rba. We conclude that the voltage and resistance changes produced by choroid-to-retina current originate in the RPE.

The simplest explanation for the decrease in R_total and increase in R_ap/R_ba is a decrease in R_ba. If this were to arise from an increase in the conductance for an ion with a relatively positive equilibrium potential, it could also account for the depolarization of V_ba. Two observations, however, argue against this being the only effect of choroid-to-retina current. First, due to the shunting of current, a change in the basal membrane battery will produce a voltage response at the apical membrane, and this would have depolarized V_ap; instead, a hyperpolarization was observed. The second argument comes from the finding that immediately after the onset of current the TEP (and hence TTP) was smaller than it was before current onset. In the open circuit,

\[
\text{TEP} = (V'_{ba} - V'_{ap}) \left[ \frac{R_s}{R_{sp} + R_{ba} + R_s} \right]
\]

where V'_{ba} and V'_{ap} are the equivalent batteries of the apical and basal membranes. If the only effect of choroid-to-retina current were to decrease R_ba and depolarize V_ba, then equation (2) indicates that, immediately after the offset of current, TEP should be larger than it was before current onset. Contrary to this expectation, TEP was smaller at this point in time (Figs. 4A, 5A). Thus, although a decrease in R_ba cannot be excluded, it is clear that choroid-to-retina current has additional effects.

One mechanism that could account for both the decrease in R_total and the decay of the current-induced voltage deflections is a decrease in R. The fraction of extrinsic current that flows across the basal and apical membranes in series (I_c) is given by:

\[
I_c = I_I \left[ \frac{R_s}{R_{sp} + R_{ba} + R_s} \right]
\]
where \( I_\text{t} \) is the transtissue current and \( I_\text{c} \) is the transcellular current. The transcellular component of extrinsic current produces voltage drops across the apical and basal membranes:

\[
\Delta V_{\text{ap}} = I_\text{c} \cdot R_{\text{ap}} \quad (4)
\]
\[
\Delta V_{\text{ba}} = I_\text{c} \cdot R_{\text{ba}} \quad (5)
\]

A gradual decrease in \( R_{\text{ba}} \) would decrease \( I_\text{c} \) (equation 3), leading to a decline in the IR drops across the basal and apical membranes (equations 4 and 5). Since both \( V_{\text{ba}} \) and \( V_{\text{ap}} \) changed slowly toward their preclamp values after undergoing initial voltage deflections (Figs. 4A, 5A), it seems likely that choroid-to-retina current causes a decrease in \( R_{\text{c}} \). The increase in \( R_{\text{ap}}/R_{\text{ba}} \) suggests the possibility of a decrease in \( R_{\text{ba}} \) or an increase in \( R_{\text{ap}} \), but it could also be a consequence of resistance changes in the paracellular pathway.

**Effect of Choroid-to-Retina Current on C-Wave Amplitude**

To gain additional information about the nature of the resistance changes produced by choroid-to-retina current, we monitored changes in the intracellular responses that underlie the c-wave in the retina-RPE-choroid preparation. The transtissue c-wave originates from a photoreceptor-induced decrease in subretinal K+ concentration that hyperpolarizes the RPE owing to the large K+ conductance of the apical membrane and also hyperpolarizes the Muller cell to give slow PII, the neural retina component. The RPE c-wave represents the difference between the hyperpolarization of the apical membrane and the hyperpolarization of the basal membrane, the latter of which is generated by the shunting of current from the apical membrane. The following equations are relevant to the voltage changes that underlie the RPE component of the c-wave and have been derived previously:

\[
\Delta V_{\text{ba}} = \Delta V'_{\text{ap}} \left[ \frac{R_{\text{ba}}}{R_{\text{ap}} + R_{\text{ba}} + R_{\text{s}}} \right] \quad (6)
\]
\[
\Delta V_{\text{ap}} = \Delta V'_{\text{ap}} \left[ \frac{R_{\text{ba}} + R_{\text{s}}}{R_{\text{ap}} + R_{\text{ba}} + R_{\text{s}}} \right] \quad (7)
\]
\[
\Delta \text{TEP} = \Delta V_{\text{ba}} - \Delta V_{\text{ap}} \quad (8)
\]
\[
\Delta \text{TP} = \Delta \text{TEP} - \Delta V_{\text{t}} \quad (9)
\]

where \( \Delta V'_{\text{ap}} \) is the change in apical membrane battery resulting from the change in subretinal K+ concentration. If any of the resistance parameters were to change slowly with time, then the amplitude of the voltage responses at the apical and basal membranes would change in a predictable fashion. For example, if \( R_{\text{ba}} \) were to decrease, then equations (6) and (7) predict smaller hyperpolarizations of \( V_{\text{ba}} \) and \( V_{\text{ap}} \) with the decrease in \( V_{\text{ap}} \) being smaller than the decrease in \( V_{\text{ba}} \). This would increase the amplitude of the RPE c-wave (equation 8). A decrease in \( R_{\text{ba}} \), however, lead to a larger hyperpolarization of \( V_{\text{ba}} \) and a smaller hyperpolarization of \( V_{\text{ap}} \), decreasing the c-wave amplitude.

Figure 6A shows the transtissue c-wave (top), slow PII, and RPE c-wave measured before and during the passage of \(-15 \mu\text{A} \) current across a retina-RPE-choroid preparation. In this figure, the polarity of \( V_{\text{c}} \) (slow PII) was inverted such that, like the TEP (RPE c-wave, it is shown retina-side positive. Choroid-to-retina current reversed the polarity of the transtissue c-wave, and this change was due to a decrease in the amplitude of the RPE c-wave (equation 9). The size of slow PII was unchanged, indicating that the magnitude of the light-evoked decrease in subretinal K+ concentration was not altered by choroid-to-retina current clamp. Figure 6B shows the effect of \(-15 \mu\text{A} \) current on the intracellularly recorded RPE c-wave. It decreased in amplitude during choroid-to-retina current clamp because of an increase in size of the hyperpolarization at the basal membrane and a decrease in the size of the hyperpolarization at the apical membrane. (This change in the \( V_{\text{ap}} \) hyperpolarization must have originated at the apical membrane since slow PII was unchanged.) Similar results were obtained in one other experiment. These findings provide additional support for the hypothesis that choroid-to-retina current caused a decrease in \( R_{\text{c}} \).

**Discussion**

Our results indicate that the basal membrane of the chick RPE lacks a conductance that is either activated by depolarization or deactivated by hyperpolarization in the physiologic voltage range. Evidence for this conclusion came from the finding that depolarizing the \( V_{\text{ba}} \) by 15 mV or less with extrinsic current in the retina-to-choroid direction had essentially no effect on either \( R_{\text{ap}}/R_{\text{ba}} \) or \( R_{\text{Total}} \). In contrast, hyperpolarizing \( V_{\text{ba}} \) by 15 mV with choroid-to-retina current led to a gradual decrease in \( R_{\text{Total}} \) and an increase in \( R_{\text{ba}}/R_{\text{s}} \) that developed with a half-time of about 1 min. Analysis of accompanying changes in membrane voltages and changes of intracellular c-wave amplitude suggested that the most likely cause of the decrease in \( R_{\text{Total}} \) was a decrease in the resistance of the paracellular pathway.
Fig. 6. Effect of choroid-to-retina current clamp on the c-wave. (A) Changes in transretinal (slow PIII) and transepithelial (RPE c-wave) components of the c-wave recorded before and during -15 μA current clamp. Light stimulus (1.8 × 10⁻⁵ W/cm²) was 4 sec in duration. (B) Effect of negative current clamp -15 μA on intracellularly recorded c-wave.

lar shunt pathway. The change in R<sub>ap</sub>/R<sub>ba</sub> could reflect an increase in R<sub>ap</sub> or a decrease in R<sub>ba</sub> or both. We will consider the origin of this effect and the significance of our findings for light-evoked RPE responses.

Several lines of evidence indicate that the decrease in R<sub>total</sub> most likely reflects a decrease in R<sub>a</sub>. After a voltage deflection at the onset of current clamp, V<sub>ap</sub> and V<sub>ba</sub> repolarized toward their preclamp values with time courses that were similar to those of the changes in R<sub>total</sub> and R<sub>ap</sub>/R<sub>ba</sub> (Fig. 4). These voltage changes cannot be explained solely by a decrease in R<sub>ba</sub> or by a change in the extrinsic current that traversed the transcellular pathway, as would occur in the face of a decrease in R<sub>a</sub>. Additional evidence for a decrease in R<sub>a</sub> came from intracellular measurements of the c-wave (Fig. 6B), which showed that choroid-to-retina current clamp decreased the size of the V<sub>ap</sub> hyperpolarization and increased the size of the V<sub>ba</sub> hyperpolarization. These changes are consistent with a decrease in R<sub>a</sub> but are incompatible with a decrease in R<sub>ba</sub>. Hence, it is likely that the decrease in R<sub>total</sub> results, at least in part, from a decrease in R<sub>a</sub>.

**Mechanism of the Current-Induced Decrease in R<sub>a</sub>**

In other epithelia, a decrease in R<sub>a</sub> originates from a current-induced change in the paracellular pathway. For instance, in the gallbladder, a tall columnar epithelium, current-induced decreases in R<sub>a</sub> were attributed to changes in the fluid conductivity and/or width of the lateral intercellular spaces that are present on the basal side of the apically located tight junctions. These intercellular space changes are thought to result from the polarization of salt in the unstirred layers adjacent to the epithelium due to the transport-number effect.

In the chicken RPE, the tight junction is located close to the basal membrane such that the major portion of the lateral intercellular space is located apically and is contiguous with the subretinal space that lies outside the apical membrane. Since the apical processes extend to the inner segments of the photoreceptors, this lateral intercellular space is relatively long. It is conceivable, then, that changes in the resistance of the lateral and subretinal spaces cause the observed changes in R<sub>a</sub>. Although these experiments
do not allow us to eliminate the possibility of a decrease in the resistance of the tight junction, the magnitude of the choroid-to-retina-induced changes in membrane voltage and resistance parameters were smaller in the absence of the neural retina than in its presence (Figs. 4, 5), suggesting, perhaps, the involvement of the subretinal space.

Previous studies show that the paracellular pathway in frog RPE is Na⁺ selective. If the paracellular shunt pathway in chick RPE is similarly Na⁺ selective, then choroid-to-retina current should deplete Na⁺ from the choroid and accumulate it in the subretinal space due to the transport-number effect. The resulting osmotic water movements would tend to expand the subretinal space, decreasing its resistance. The results of preliminary experiments involving the superfusion of the basal side of the retina–RPE–choroid with a Na⁺-free solution (N-methyl-D-glucamine substitution) supported this hypothesis as follows: (1) basal Na⁺ removal hyperpolarized the basal membrane and depolarized the apical membrane, consistent with a Na⁺-selective paracellular pathway and (2) in the absence of basal Na⁺, the time-dependent changes in membrane voltage and resistance normally produced by choroid-to-retina current were eliminated completely (not shown).

The origin of the choroid-to-retina current-induced increase in \( R_{ap}/R_{ba} \) is unknown. Since the slow changes in \( R_{Total} \) and \( R_{ap}/R_{ba} \) follow the same time course, they probably are related casually. One possible explanation for this is that the change in the apparent ratio of membrane resistances is a consequence of a change in intercellular resistance. Equivalent circuit models of the RPE generally represent the paracellular pathway as a single resistor, combining the resistances of the tight junction and lateral intercellular space. This view may be unrealistic because a substantial fraction of \( R_s \) may lie in the resistance of the lateral and/or subretinal spaces. A more accurate model would represent the lateral membrane and subretinal space as a distributed resistive network. If this model held true for the chick RPE, then in principle, a decrease in the resistance of the lateral intercellular space might lead to an apparent decrease in \( R_{ap} \) and, hence, a decrease in \( R_{ap}/R_{ba} \). However, we found that the decrease in \( R_s \) was associated with an increase in \( R_{ap}/R_{ba} \), suggesting a different mechanism. It seems likely, therefore, that the increase in \( R_{ap}/R_{ba} \) is caused either by an increase in \( R_{ap} \) or by a decrease in \( R_{ba} \) or both. This conclusion is also supported by the observation that the increase in \( R_{ap}/R_{ba} \) was present in the isolated RPE–choroid, where the neural retina no longer delimits the subretinal space and the resistance of the lateral intercellular space is expected to be lower. Although our experiments do not allow us to distinguish between these possibilities, it is worthwhile pointing out that whole-cell patch-clamp studies in isolated RPE cells of frog and rat show the presence of an inwardly rectifying K⁺ conductance. If this channel were present on the apical membrane, as has been suggested for the frog RPE, then the conductance of this membrane would be expected to decrease as \( V_{ap} \) is depolarized by choroid-to-retina current, leading to the observed increase in \( R_{ap}/R_{ba} \).

Implications for the Origins of the Fast Oscillation and Light Peak

The fast oscillation and light peak are two components of the DC ERG that originate as voltage changes of opposite polarity across the RPE basal membrane. In addition, these changes in \( V_{ba} \) seem to be associated with changes in the resistance of that membrane; the apparent resistance of the basal membrane increases as \( V_{ba} \) hyperpolarizes during the fast oscillation, and it decreases as the membrane depolarizes during the light peak. In addition, other experimental manipulations that depolarize \( V_{ba} \) such as hypoxia, retinal hyperosmolarity, choroidal azide, and retinal dopamine are associated with a decrease in \( R_{ba} \); whereas those that hyperpolarize it are associated with an increase in \( R_{ba} \). These findings raised the question of whether the basal membrane is voltage sensitive in the physiologic range. For the light-evoked responses, the question is whether the resistance changes associated with the fast oscillation and light peak are a cause or a consequence of the mechanism generating these responses. A voltage-sensitive conductance necessary to account for these resistance changes would be expected to be outwardly rectifying near the resting potential of the basal membrane. Our observation that the basal membrane lacks a voltage-sensitive conductance in the physiologic voltage range suggests that the conductance changes that accompany the fast oscillation and light peak most likely result from either changes in intracellular ion concentration or the modulation of channels by second messengers.

Key words: retina, pigment epithelium, fast oscillation, light peak

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References