Modification by Cyclic Adenosine Monophosphate of Basolateral Membrane Chloride Conductance in Chick Retinal Pigment Epithelium

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Purpose. We investigated the hypothesis that cyclic adenosine monophosphate (cAMP) modifies the basolateral membrane chloride conductance of chick retinal pigment epithelium (RPE).

Methods. Placing freshly dissected chick retina-RPE-choroid tissues in a perfusion chamber that allows separate perfusion of its retinal and choroidal sides, the authors examined the effect of choroidal perfusion of forskolin (50 mM), an adenylate cyclase activator, on extracellular and intracellular RPE potentials, resistances, light-evoked responses, and chloride diffusion potentials.

Results. Forskolin hyperpolarized the RPE basolateral membrane, decreased the apical/basal membrane resistance ratio, increased the amplitude of the RPE membrane c-wave hyperpolarizations, decreased the amplitude of the transepithelial c-wave, and decreased the light peak. In addition, forskolin decreased the basolateral membrane chloride diffusion potential by 31%. The effects of forskolin were diminished by pretreatment of the basolateral membrane with DIDS, a chloride channel blocker, or by pretreatment of the apical membrane with bumetanide, a blocker of NaK2Cl cotransport. Transepithelial potential, resistance, and c-wave amplitude were not changed by 1,9-dideoxyforskolin, which does not elevate cAMP levels.

Conclusions. Elevation of cAMP results in diminished basolateral membrane chloride conductance in chick RPE. This could be due to a direct effect on the chloride channel or due to a decrease in intracellular chloride concentration secondary to inhibition of apical membrane NaK2Cl cotransport. Invest Ophthalmol Vis Sci. 1994;35:422-433.

The retinal pigment epithelium (RPE) regulates fluid and ion transport between the neural retina and the choroidal circulation. Although the mechanisms of transepithelial water movement are not completely understood, the transport of chloride from the retina to the choroid may play an important role.1,2 These functions of the RPE are believed to be vital for normal retinal activity, retinal adhesion, and especially for the survival of photoreceptors.

Intracellular chloride is maintained above its equilibrium concentration by a NaK2Cl cotransporter in many epithelia.3 This has been observed in toad4 and bovine5 RPE. In chick RPE, intracellular chloride is maintained above its equilibrium concentration by a furosemide-inhibitable mechanism on the apical membrane, probably a NaK2Cl cotransporter,6,7 and flows out across a basolateral membrane chloride channel.8,9 Modulation of the apical membrane NaK2Cl cotransporter or basolateral membrane chloride channel would enable the RPE to control chloride transport from the retina to the choroid, and provide a mechanism for controlling the movement of fluid out of the subretinal space.

In many epithelia, chloride transport is regulated by one or more second messengers, including cyclic adenosine monophosphate (cAMP).10,11 In the freshly isolated frog RPE-choroid preparation, cAMP diminishes chloride transport across the RPE,12-15 decreases
apical membrane NaK2Cl cotransport and increases basolateral membrane chloride conductance. cAMP increases chloride conductance in isolated toad and bullfrog RPE cells. In cultured monkey RPE, cAMP decreases NaK2Cl cotransport.

Exogenous cAMP was shown to increase chick RPE basolateral membrane resistance and hyperpolarize the basolateral membrane in previous work from this laboratory. In the current experiments we explored the hypothesis that these earlier findings could be explained by a decrease in basolateral membrane chloride conductance. Using the in vitro chick retina–RPE–choroid preparation, we investigated the effect of forskolin on RPE potential, resistance, and light-evoked responses. Forskolin increases intracellular cAMP levels by reversibly activating adenylate cyclase. We used chloride diffusion potentials, the light peak, 4,4'diisothiocyanatostilben-2,2'-disulfonic acid (DIDS, a chloride channel blocker), and bumetanide (an inhibitor of NaK2Cl cotransport) to investigate the effect of forskolin on RPE chloride conductance. Our results indicate that cAMP decreases RPE basolateral membrane chloride conductance. Portions of this work have appeared in abstract form.

METHODS

Preparation

1 to 10 day old chicks (Gallus domesticus) were light adapted for two hours, then dark adapted for 5 minutes before decapitation. All chicks were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Under dim red light eyes were excised and mounted, cornea down, beneath the surface of a bath containing control solution (described below) at 37°C, bubbled before dissection with 5% CO2/95% O2 gas to maintain pH at 7.45 ± 0.15. A 9-mm-diameter circular region of sclera was cut away without disturbing the choroid. Using a razor blade, a small incision through the choroid, RPE, and retina was made near the optic nerve. Avoiding the optic nerve, a 6-mm-diameter circular region of retina–RPE–choroid was cut free from the eye using retina scissors and placed, choroid down, on a fine mesh made of 37-nm nylon thread with 50% open area. This preparation was positioned between two Lucite plates, which were pressed together using screws, to form a modified Ussing chamber. Each plate had a small circular hole, exposing a tissue area of 7.0 mm2. The apical (retinal) and basal (choroidal) sides of the tissue were perfused separately. The basal flow rate was 1.2 to 1.4 ml/min into a chamber volume of 0.1 ml, allowing an exchange of 12 to 14 chamber volumes per minute. The apical flow rate was 1.5 ml/min. This entire system was encased in a light-tight box.

Solutions

The tissue was perfused with a gravity-fed system. The control perfusate (120 mM NaCl, 5 mM KCl, 25 mM NaHCO3, 25 mM dextrose, 2.5 mM CaCl2, 3.7 mM MgCl2; all reagents from Sigma, St. Louis, MO) was contained in a closed 1-l reservoir, where it was warmed to 37°C and bubbled constantly with 5% CO2/95% O2 to maintain a pH of 7.45 ± 0.15. Just before reaching the tissue, the solution was passed through another set of heating coils to maintain a constant tissue temperature of 36°C. The osmolarity of this solution was 308 ± 8 mOsm (model 5500 Vapor Pressure Osmometer, Wescor, Inc., Logan, UT). The relatively high concentration of magnesium was used because it reduces spreading depression in chick retina. Chick retina is prone to spreading depression, and this phenomenon has been studied previously.

Forskolin and 1,9-dideoxyforskolin (Calbiochem, San Diego, CA) were dissolved in DMSO (Sigma) to a concentration of 100 mM, then diluted in control perfusate to a final concentration of 50 mM forskolin or 1,9-dideoxyforskolin in 0.05% DMSO.

Solutions containing 100 mM DIDS (Sigma) were prepared by dissolving DIDS directly in warm (50°C) perfusate, then stirring constantly for 30 minutes. DIDS solutions were maintained in the dark or under dim red light because DIDS is light sensitive. The solution was cooled to 37°C before using. Solutions containing 0.5 mM bumetanide (Sigma) were prepared in exactly the same manner.

Chloride diffusion potentials were obtained by replacing NaCl with NaMeSO4 (Aldrich, Milwaukee, WI), reducing chloride concentration by 87.3%, from 137.4 to 17.4 mM.

All solutions were identically heated and bubbled. The osmolarity and pH of these solutions were not significantly different from those of the control solution (within 8 mOsm and 0.15 pH units).

Electrodes

Conventional electrodes were pulled from Omega Dot glass tubing, 1.0 mm OD and 0.76 mm ID on a horizontal glass puller (model P-77, Sutter Instruments, San Francisco, CA). Electrodes were backfilled with 250 mM KCl for intracellular recording and 100 mM NaCl/10 mM KCl for extracellular recording. A silver chloride wire was placed in each electrode before sealing with wax. Electrodes were used without beveling for intracellular and extracellular recording, and had resistances of 100 to 200 Ω in control perfusate. Microelectrode and apical bath potentials were measured using unity gain amplifiers with input resistances of 1014 Ω (model 1090, Winston Electronics, San Francisco, CA).
Recording Configurations

Electrodes were used to record intracellularly in the RPE and extracellularly in the subretinal space and subepithelial space. RPE impalements were identified by a sudden drop in potential of 60 to 70 mV in association with appropriate changes in resistance and changes in light-evoked responses. Subretinal recordings were obtained by withdrawing the microelectrode 24 to 30 mm from the RPE, and subepithelial recordings were obtained by advancing the microelectrode 10 to 30 mm beyond the RPE, using a precision microelectrode manipulator (Burleigh 6000, Fishers, NY). Measurements of voltage and resistance with respect to the basal bath were used to verify the position of the microelectrode in the subretinal or subepithelial space.

For experiments in which chloride changes were made in the choroidal bath, junction potentials occurred at the basal agar bridge and across the choroid, both of which have been previously described. The combination of the basal agar bridge junction potential and the choroidal potential was identified by positioning a microelectrode backfilled with 3 M KCl in the subepithelial space and reducing the chloride concentration in the basal bath by the same amount and duration as in the experiment. Using this method, a junction potential of approximately -4 mV was consistently identified. This trace, containing only the junction potentials and not the tissue response, was subtracted from Vba and transtissue recordings to reveal the actual changes in tissue potential.

Equivalent Electrical Circuit

Figure 1 demonstrates the equivalent electrical circuit for the RPE. Transtissue potential was measured using 500 mM KCl agar bridges placed in the apical and basal baths (positions 1 and 5, respectively). With a microelectrode in the subretinal space (position 2), potentials across the retina and RPE-choroid were measured by referencing to the apical and basal bath agar bridges, respectively. Intracellular recordings (position 3) revealed the sum of the RPE apical membrane and transtissue potential when referenced to the apical bath, and the sum of the RPE basal membrane and choroidal potential when referenced to the basal bath.

To measure resistance, 3-μA currents were passed across the tissue for 4 seconds at intervals of 40 to 45 seconds using an automated current passing system (model VCC600, Physiologic Instruments, San Diego, CA). The resulting brief changes in potential were measured and resistance calculated using Ohm's Law. The availability of a shunt current (Rs in Fig. 1) complicated direct measurements of apical and basolateral membrane resistances. Instead, the apical/basal membrane resistance ratio was calculated from the brief current-induced changes in RPE apical and basal membrane potential. No correction was made for the voltage drops that occurred across the retina or choroid, because these did not change over the course of the experiments and therefore did not interfere with our observations.

Taking advantage of the functioning retina in this
system, light-evoked RPE responses were generated by exposing the tissue to diffuse white light from a halogen lamp at 60 mW/cm². c-Waves were elicited with 4-second light exposures at 80- to 90-second intervals, and light peaks were elicited with 5-minute exposures. Changes in c-wave amplitude were used to confirm changes in RPE membrane resistance using the following equations, derived previously, which demonstrate that the extracellular and intracellular potential changes of the c-wave are functions of RPE apical membrane, basolateral membrane, and shunt resistances.²⁵ (see Ref. 25 for complete derivations).

\[
\Delta V_{ap} = \Delta V'_{ap} \left( \frac{R_{h,b} + R_s}{R_{ap} + R_{h,b} + R_s} \right)
\]

(1)

\[
\Delta V_{lb} = \Delta V'_{lb} \left( \frac{R_{h,b}}{R_{ap} + R_{h,b} + R_s} \right)
\]

(2)

\[
\Delta TEP = \Delta V_{lb} - \Delta V_{ap}
\]

(3)

\[
\Delta TEP = -\Delta V'_{ap} \left( \frac{R_s}{R_{ap} + R_{h,b} + R_s} \right)
\]

(4)

where \( V_{ap} (V_{lb}) \) = apical (basolateral) membrane potential, \( V'_{ap} (V'_{lb}) \) = apical (basolateral) membrane battery; \( TEP \) = transepithelial potential, and \( R_{ap}, R_{h,b}, \) and \( R_s \), are the apical membrane, basolateral membrane, and shunt resistances, respectively.

All recorded signals were stored digitized (8 Hz) as a Lotus 123 spreadsheet using Labtech Notebook (Scientific Solutions, Wilmington, MA). All figures were made with SigmaPlot (Jandel Scientific).

**RESULTS**

**Control Electrical Parameters**

Thirty to 60 minutes after mounting, tissues tended to stabilize at an average transtissue potential of 5.40 ± 1.20 mV and transtissue resistance of 1.95 ± .52 kΩ (n = 17 tissues) (all values given as mean ± standard deviation). Potential and resistance then remained stable for 2 to 5 hours.

**Effect of Forskolin on Membrane Potential**

We investigated the effect of forskolin on transtissue potential by perfusing the basal (choroidal) side of the tissue with 50 mM forskolin for 8 minutes. We have found that the choroid in this preparation is reasonably permeable to small molecules such as forskolin (MW 410), and chose to perfuse the choroidal side of the tissue to minimize the effect of forskolin on the neural retina. Figure 2 demonstrates that forskolin decreased the transtissue potential by diminishing its transepithelial component, whereas the tranretinal potential was not significantly affected. In 10 experiments, we observed an average reversible decrease in transepithelial potential of 1.89 ± 0.47 mV. The transepithelial potential stabilized at a new, lower potential, within 5 minutes after forskolin exposure.

The normal resting potential of the RPE basolateral membrane is depolarized relative to that of the apical membrane, resulting in the retina-positive transepithelial potential. Any change in the transepithelial potential is a manifestation of a change in one or both of the RPE membrane potentials. To determine whether the change in transepithelial potential originated at the RPE apical or basolateral membrane, we obtained intracellular recordings of the RPE as shown in Figure 3. In every case, choroidal forskolin perfusion hyperpolarized the basolateral membrane (−3.2 ± 1.1 mV, n = 6), and this was accompanied by a smaller apical membrane hyperpolarization, −2.00 ± 1.08 mV. That the basolateral membrane hyperpolarization was larger than the apical indicated that the hyperpolarization originated at the basolateral membrane.²⁶ The apical membrane hyperpolarization was partially or fully a passive event driven by a change in the shunt current due to the basolateral membrane hyperpolarization.

The hyperpolarization of the RPE basolateral membrane could be attributed to either of two possible membrane conductance changes; an increase in conductance to an ion with an equilibrium potential that is negative relative to the membrane resting potential, or a decrease in conductance to an ion with an equilibrium potential that is positive to the membrane resting potential. To explore these possibilities, we investigated the effect of forskolin on three measures of resistance across the tissue; the transtissue resistance,
Transtissue Potential

FIGURE 3. The effect of choroidal forskolin perfusion on the transtissue potential and RPE apical ($V_{ap}$) and basolateral ($V_{ba}$) membrane potentials. The perfusate was changed to the test solution containing forskolin (bar). The hyperpolarization of the basolateral membrane accounts for the decrease in the transtissue potential. The smaller apical membrane hyperpolarization was completely or in part a passive event driven by the basolateral membrane hyperpolarization. The transients are c-waves.

The increase in transepithelial resistance and the RPE apical/basal membrane resistance ratio. Figure 4 demonstrates that forskolin reversibly increased the transtissue resistance, which is the sum of the transepithelial and transretinal resistances. By recording from a microelectrode placed in the subretinal space, we demonstrated that the resistance across the retina did not change significantly, but the transepithelial resistance increased, accounting for the increase in transtissue resistance. In ten tissues, the transepithelial resistance increased by $0.12 \pm 0.07 \Omega$, from 1.50 to 1.62 $\Omega$, with an increase in transepithelial resistance observed in every case.

The increase in transepithelial resistance could be due to an increase in RPE apical membrane resistance, basolateral membrane resistance, shunt resistance, or a combination of these (Fig. 1). To locate the origin of the resistance change, we calculated the ratio of RPE apical and basolateral membrane resistances from intracellular recordings. Figure 4 demonstrates that forskolin reduced the apical/basal membrane resistance ratio with a time-course identical to those of the increase in transtissue resistance and the basolateral membrane hyperpolarization. In eight tissues, an average decrease in the apical/basal membrane resistance ratio of $0.034 \pm 0.028$ was observed, from 0.245 to 0.211. The simplest explanation for an increase in transepithelial resistance and a decrease in the apical/basal membrane resistance ratio is an increase in RPE basolateral membrane resistance.

The transepithelial resistance could be due to an increase in RPE basolateral membrane resistance, shunt resistance, a combination of these (Fig. 1). To locate the origin of the resistance change, we calculated the ratio of RPE apical and basolateral membrane resistances from intracellular recordings. Figure 4 demonstrates that forskolin reduced the apical/basal membrane resistance ratio with a time-course identical to those of the increase in transtissue resistance and the basolateral membrane hyperpolarization. In eight tissues, an average decrease in the apical/basal membrane resistance ratio of $0.034 \pm 0.028$ was observed, from 0.245 to 0.211. The simplest explanation for an increase in transepithelial resistance and a decrease in the apical/basal membrane resistance ratio is an increase in RPE basolateral membrane resistance.

To further investigate the effect of forskolin on the RPE basolateral membrane resistance, we elicited c-waves by illuminating the tissue with diffuse white light for 4 seconds at 80- to 90-second intervals. The transepithelial c-wave is a light-evoked positive deflection in potential that peaks after about 3 seconds, and is caused by a light-induced decrease in the potassium concentration of the subretinal space that rapidly hyperpolarizes the RPE apical membrane, with a smaller, passive hyperpolarization of the basolateral membrane. Forskolin reduced the transepithelial c-wave amplitude and an increase in the basolateral membrane c-wave hyperpolarization when an increase in basolateral membrane resistance is observed (see equations in Methods). Consistent with these predictions, forskolin reduced the transepithelial c-wave amplitude by $0.46 \pm 0.19 \text{mV}$, from 1.18 mV to 0.72 mV ($n = 9$) (Fig. 5A), and increased the amplitude of the RPE basolateral membrane c-wave hyperpolarization by $0.58 \pm 0.17 \text{mV}$ ($n = 6$) (Fig. 5B). The amplitude of the apical membrane c-wave hyperpolarization was increased by a smaller amount, $0.44 \pm 0.21 \text{mV}$, consistent with a passive shunt response. We observed a much smaller change in the retinal light response (transretinal in Fig. 5A).
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which implies that this aspect of retinal function was not significantly altered by forskolin. This is consistent with earlier evidence that cAMP has only a small effect on retinal light responses in this system.19

The c-wave changes produced by forskolin provided additional evidence that it reduced a basolateral membrane conductance. The time course of the changes in transepithelial c-wave amplitude was identical to those for the changes in transepithelial potential and resistance, implying that a single event was responsible for each independent observation.

30-Minute Exposure to Forskolin

These initial observations demonstrated that brief (8-minute) exposures to forskolin produced simple and repeatable effects on RPE electrophysiologic parameters. To investigate the possibility that additional, more slowly developing responses might exist, we perfused the choroidal side of the tissue with 50 mM forskolin for 30 minutes. In two tissues, we did not observe any other changes in transepithelial potential, resistance, or light-evoked responses with longer exposure (Fig. 6). In addition, Figure 6 demonstrates that potential, resistance, and c-wave amplitude transiently rebounded beyond control levels within 30 minutes after removal of forskolin. This rebound effect also was seen in the apical/basal membrane resistance ratio (Fig. 4) and the basolateral membrane chloride diffusion potential (Fig. 9, discussed below).

Our observations support the hypothesis that forskolin diminished the conductance of an ion that has an equilibrium potential positive to the resting membrane potential. In chick RPE, chloride is estimated to have an equilibrium potential of −40 ± 10 mV,8 and a chloride conductance on the chick RPE basolateral membrane has recently been demonstrated.9 We found baseline basolateral membrane potential to be −61.0 ± 0.86 mV (n = 6). Thus, a decrease in basolateral membrane chloride conductance would increase the basolateral membrane resistance.
and hyperpolarize the membrane, consistent with our observations. The experiments described below address the possibility that forskolin diminishes RPE basolateral membrane chloride conductance.

**DIDS**

We investigated the effect of choroidal pretreatment with DIDS on the forskolin response. We chose to use DIDS because it is an anion channel blocker that was previously shown to diminish an RPE basolateral membrane chloride conductance in this preparation. We hypothesized that pretreatment of the basolateral membrane with DIDS would diminish any forskolin-induced changes in transepithelial potential, resistance, or c-wave attributable to changes in basolateral membrane chloride conductance. As shown in Figure 7, pretreatment of the RPE basolateral membrane with 100 mM DIDS for 30 minutes substantially reduced the changes in transepithelial potential and c-wave amplitude associated with forskolin, with a smaller effect on the forskolin-induced transepithelial resistance change (see Discussion). In two experiments, DIDS reduced the average transepithelial response to forskolin by 60% (potential) and 60% (c-wave).

**Bumetanide**

To explore a possible suppression of RPE apical membrane NaK2Cl cotransport by forskolin, we blocked the cotransporter with bumetanide before perfusing with forskolin. As shown in Figure 8, perfusing the apical bath with 0.5 mM bumetanide for 32 minutes reduced the changes in transepithelial potential, c-wave, and resistance associated with forskolin. In three tissues, we found that bumetanide diminished the effect of forskolin on transepithelial potential, resistance, and c-wave amplitude by 21%, 12%, and 24%, respectively.

**Light Peak**

When the dark-adapted retina of many species, including chick, is illuminated for several minutes, a gradual increase in the RPE transepithelial potential oc-
FIGURE 9. Effect of choroidal forskolin perfusion on the transepithelial light peak. Light peaks were obtained by illuminating the tissue with 60-mW/cm² diffuse white light for 5 minutes. Forskolin was perfused for 10 minutes before the forskolin light peak was obtained. The tissue was allowed to dark-adapt for at least 25 minutes before each period of illumination.

curs, termed the light peak. The light peak was recently shown to be mediated by an increase in basolateral membrane chloride conductance in chick. We investigated the effect of forskolin on the light peak by illuminating the tissue for periods of 5 minutes, allowing 25 minutes for dark adaptation between illuminations. After obtaining a control light peak, we perfused the choroidal side of the tissue for 8 minutes with 50 mM forskolin. While continuing to perfuse with forskolin, we obtained a second light peak. Figure 9 demonstrates that forskolin profoundly suppressed the transepithelial light peak. A substantial and progressive recovery was observed at 15 and 45 minutes after return to control solution. In two experiments, control, forskolin, and 45-minute recovery light peaks averaged 0.89, 0.05, and 0.38 mV, respectively.

Diffusion Potentials

A more specific method for investigating the conductance to chloride in this system is to record the membrane-potential change elicited by altering extracellular chloride concentration (diffusion potential). If the basolateral membrane chloride conductance is diminished, then the diffusion potential to chloride also will

FIGURE 10. Effect of choroidal forskolin perfusion on the RPE basolateral membrane chloride diffusion potential. The extracellular chloride concentration was lowered from 137.4 to 17.4 mM for 3 minutes. The control recording was obtained before forskolin perfusion, and the forskolin recording was obtained after 5 minutes of choroidal forskolin perfusion.
be reduced. We elicited basolateral membrane chloride diffusion potentials by reducing the chloride concentration in the choroidal bath for 3-minute periods by 87%, from 157.4 to 17.4 mM, while recording intracellularly. As seen in Figure 10, forskolin reversibly reduced the basolateral membrane chloride diffusion potential. In five experiments (four tissues), the diffusion potential was decreased by 31 ± 15%, with a reduction in the diffusion potential seen in every case. In all of the diffusion potential experiments, a small apical membrane hyperpolarization accompanied the basolateral membrane depolarization (not shown). This resulted from a shunt current secondary to decreased shunt permeability to the substitute anion previously observed in chick, that was difficult to measure accurately. Thus, these diffusion potentials included a small contribution from a paracellular shunt potential, as previously observed in chick, that was difficult to measure accurately.

**Control Experiments**

1,9-dideoxyforskolin, an analog of forskolin, does not elevate cAMP but otherwise mimics most of the non-cAMP-dependent effects forskolin might have. Specifically, forskolin has been reported to decrease a chloride conductance by a non-cAMP-dependent mechanism, which can be revealed by 1,9-dideoxyforskolin. Fifty millimolar 1,9-dideoxyforskolin perfused in the choroidal bath for 10 minutes did not produce any significant changes in transepithelial potential, resistance, or c-wave (n = 2). This implies that the forskolin-induced changes we observed could be attributed to an increase in intracellular cAMP.

**DISCUSSION**

Using the chick retina–RPE–choroid preparation we demonstrated that choroidal forskolin perfusion reversibly increased the transepithelial resistance, decreased the RPE apical/basal membrane resistance ratio, decreased the transepithelial c-wave, and increased the amplitude of the intracellular c-wave hyperpolarization. All of these observations are consistent with an increase in basolateral membrane resistance. We also found that forskolin decreased the transepithelial potential by hyperpolarizing the RPE basolateral membrane. No changes in transepithelial potential, resistance, or c-wave amplitude were observed with 1,9-dideoxyforskolin, which argues against direct action of forskolin and implies that the effects observed occurred secondary to elevation of intracellular cAMP.

A decrease in basolateral membrane chloride conductance would suffice to hyperpolarize the membrane, because the equilibrium potential of chloride is positive to the resting membrane potential. It is not clear from our data alone that a decrease in chloride conductance was the primary event, because the hyperpolarization of the basolateral membrane by some other mechanism could, in the presence of a voltage-sensitive chloride channel, lead to a decrease in chloride conductance. Recent evidence in chick indicates, however, that the RPE basolateral membrane chloride conductance is not voltage sensitive. On this basis we propose that forskolin diminished an RPE basolateral membrane chloride conductance, resulting in a hyperpolarization of the basolateral membrane. We tested this hypothesis by observing the effect of forskolin on the light peak and the basolateral membrane chloride diffusion potential, and by pretreating the basolateral membrane with the chloride channel blocker DIDS.

We found that forskolin profoundly and reversibly suppressed the light peak, which has been demonstrated, in chick, to be mediated by an increase in RPE basolateral chloride conductance. Although this finding is consistent with the hypothesis that forskolin (and therefore elevated cAMP) diminishes this conductance, a number of conditions and agents will inhibit the light peak and the chain of intracellular and extracellular events underlying the light peak are not well understood. It has been proposed that a light-evoked diffusible substance derived from the photoreceptor alters the activity of a second messenger within the RPE cell, leading to the increase in basolateral membrane chloride conductance. cAMP was previously rejected as a candidate for the light peak second messenger in chick, in part because the effects of cAMP on RPE potential and resistance were opposite to those observed during the light peak. The putative light-evoked diffusible substance, however, may have an inhibitory effect on a second messenger system within the cell, rather than an excitatory effect, or the RPE second messenger could be elevated by a photoreceptor-derived substance secreted by the dark-adapted retina. The light peak, therefore, still could be due to a light-evoked suppression of a second messenger effect in the RPE, as has been suggested in the past, and cAMP could be the messenger.

We found that pretreatment of the RPE basolateral membrane with DIDS, a chloride channel blocker, suppressed the effects of forskolin on transepithelial potential and c-wave amplitude, but with only a small effect on transepithelial resistance. That DIDS blunted the forskolin-induced potential and c-wave changes was expected, but the persistent effect of forskolin on transepithelial resistance in the presence of DIDS requires an explanation. The predominant conductances on the RPE basolateral membrane are to potassium and chloride. It could be argued that forskolin diminishes a potassium conductance, and that this effect persists in the presence of DIDS. Di-
minishing a basolateral membrane potassium conductance would be expected to increase the transepithelial potential by depolarizing the basolateral membrane. In contrast, we observed a decrease in transepithelial potential when forskolin was added to the tissue in the presence of DIDS. It is therefore unlikely that forskolin affected the basolateral membrane potassium conductance. Instead, there is evidence that multiple chloride conductances exist on the RPE basolateral membrane, only a subset of which are sensitive to cAMP. DIDS, too, may affect some, but not all, RPE chloride conductances. It is possible, then, that forskolin continues to produce a change in basolateral membrane resistance in the presence of DIDS because DIDS affects more than one conductance, including a fraction of the cAMP-sensitive chloride conductance.

Our finding that forskolin diminishes the RPE basolateral membrane diffusion potential by 31% constitutes our most specific evidence that forskolin reduces a basolateral membrane chloride conductance. The partial persistence of the chloride diffusion potential in the presence of forskolin provides an indication that multiple chloride conductances may exist that differ in their sensitivity to cAMP.

All of the above data are consistent with the hypothesis that cAMP diminishes basolateral membrane chloride conductance in chick RPE. Chloride channels sensitive to cAMP are common in epithelia, and although cAMP most often increases chloride conductance, diminished chloride channel conductance also has been observed. There is, however, an alternative hypothesis that deserves consideration. For any of the chloride channels, a reduction in intracellular chloride concentration would decrease the apparent basolateral membrane chloride conductance by diminishing the available charge-carrier (Goldman-Hodgkin-Katz rectification). Chloride is maintained well above its equilibrium concentration in chick RPE by an apical membrane furosemide-sensitive mechanism, probably a NaK2Cl cotransporter. Inhibition of this transport would reduce the intracellular chloride concentration, as chloride continued to flow passively out of the basolateral membrane channel. Consistent with this, apical membrane perfusion with furosemide, an inhibitor of the cotransporter, increases transepithelial resistance, decreases the apical/basal membrane resistance ratio, decreases transepithelial potential, and hyperpolarizes the basolateral membrane in chick. cAMP modulates NaK2Cl cotransport in many epithelia, and both enhanced and suppressed transport have been described.

To explore a possible suppression of NaK2Cl cotransport by forskolin, we blocked the cotransporter with bumetanide before perfusing with forskolin. We found that bumetanide diminished the effect of forskolin on transepithelial potential, resistance, and c-wave amplitude, which may indicate that the apical membrane NaK2Cl cotransporter is sensitive to cAMP. It is also possible, however, that bumetanide altered intracellular volume and ionic composition, and in doing so rendered the cell less responsive to cAMP. Bumetanide can also inhibit Cl-/HCO3- exchange at concentrations used in these experiments. A Cl-/HCO3- exchanger has been demonstrated on the basolateral membrane of the bovine RPE, and could help modulate chloride concentrations.

It is important to appreciate the degree to which the apical membrane NaK2Cl cotransporter and basolateral membrane chloride channel are interrelated, not just with respect to the transport of chloride, but also with respect to the changes in electrophysiologic parameters that occur when the function of either is altered. Blocking the chloride channel with DIDS and the cotransporter with bumetanide both will diminish basolateral membrane chloride conductance (the former directly and the latter by Goldman-Hodgkin-Katz rectification), resulting in an increase in basolateral membrane resistance and a basolateral membrane hyperpolarization. Because the chloride channel and the NaK2Cl cotransporter both affect electrophysiologic parameters by altering chloride conductance, blocking one of these systems will diminish the effect that blocking the other will have on membrane potential and resistance. Thus, that blocking chloride conductance with DIDS diminishes the effect of forskolin on RPE potential and resistance leaves open the possibility that forskolin acts on either the apical membrane cotransporter or the basolateral membrane chloride channel. Both of these interpretations will also explain the observation that blocking the apical membrane cotransporter with bumetanide reduces the effects of forskolin on potential and resistance. Our experiments with DIDS and bumetanide, therefore, do not localize the predominant effect of cAMP to the basolateral membrane chloride channel or the apical membrane NaK2Cl cotransporter, but they do provide supportive evidence that cAMP modulates one or more steps in the RPE chloride transport pathway. Based on our current understanding of RPE ion transport, the most likely mechanisms for the decrease in RPE chloride conductance we have observed are suppression of the apical membrane NaK2Cl cotransporter or a direct effect on a basolateral membrane chloride channel. The next goal is to identify the specific chloride transport mechanism(s) modulated by cAMP.

Key Words
retinal pigment epithelium, cyclic adenosine monophosphate, chloride channel, NaKCl cotransport, light peak
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