Effects of cAMP and IBMX on the Chick Retinal Pigment Epithelium

Membrane Potentials and Light-Evoked Responses

Nobuhisa Nao-i,* Ron P. Gallemore, and Roy H. Steinberg

cAMP is known to alter electrical measures of retinal pigment epithelial (RPE) function (standing potential and DC ERG). To locate the origin of these effects, studies were performed on in vitro preparations of chick retina-RPE-choroid and RPE-choroid, which were separately perfused with cAMP or db-cAMP on the retinal (apical) and choroidal (basal) tissue surfaces. Similar studies were performed using the phosphodiesterase inhibitor IBMX. cAMP and db-cAMP produced essentially identical results. CAMP placed in the retinal or choroidal baths hyperpolarized the RPE basal membrane, increased the apparent basal membrane resistance ($R_{ba}$), and decreased the ERG c-wave. Experiments in RPE-choroid preparations suggested that these effects of cAMP were not secondary to effects on the neural retina. Effects were observed at 1.0 μM and increased with concentration (1.0 μM–500 μM), with choroidal application producing smaller effects than retinal. The c-wave decrease with retinal cAMP was due principally to the increase in $R_{ba}$. However, the light-evoked subretinal $K^+$ ([K+]s) decrease, measured with $K^+$-specific microelectrodes, showed a small reduction that may have contributed to the c-wave change and that explained an observed decrease in slow PHA. Retinal CAMP also led to a small decrease in ERG a-wave amplitude, which in view of the effects on [K+]s, is consistent with a small reduction in the light response of photoreceptors. The CAMP-induced hyperpolarization of the RPE basal membrane may result from a decrease in basal membrane anion conductance.

The ubiquitous nature of the second messenger function of cAMP has stimulated research on its role in the retinal pigment epithelium (RPE). There is evidence that cAMP inhibits phagocytosis by cultured RPE of rat1,2 and chick,3 and that it may be the second messenger modulating dark-adaptive aggregation of melanin pigment in teleost fish.4-6 Furthermore, cAMP in frog can modulate net fluid and ion transport,7-10 alter membrane conductances and affect the rate of the Na+-K+ pump.11 All of these important functions of the RPE, namely, phagocytosis, pigment movement, and transport, are actually aspects of interactions between the RPE and the neural retina, especially the photoreceptors, that change with light and darkness.

Dawis and Niemeyer12 showed in cat that dibutyryl cAMP (480 μM) increased the standing potential and depressed the light peak of the arterially perfused cat eye, while dibutyryl cGMP had no effect. They also provided evidence that inhibition of the phosphodiesterase could decrease the amplitude of the light peak.13 The RPE is the primary source of the standing potential of the eye and its basal membrane is the origin of the light-peak voltage.14 These studies raised

---

From the Departments of Physiology and Ophthalmology, University of California, San Francisco, San Francisco, California.

* Current address: Department of Ophthalmology, Miyazaki Medical College, Miyazaki, Japan.

Supported by NIH grant No. EY-01429 (RHS), a Research to Prevent Blindness International Research Scholar Grant (NN), and a Retinitis Pigmentosa Student Fellowship (RPG).

Submitted for publication: March 15, 1989; accepted May 22, 1989.

Reprint requests: Dr. Roy H. Steinberg, Department of Physiology, S-762, University of California, San Francisco, CA 94143-0444.
the question of whether cAMP was involved in generation of the light peak as a second messenger in the retinal pigment epithelium.

The purposes of the present experiments were to examine further how cAMP alters RPE electrical activity and the light peak. We studied both the effects of cAMP (and dibutyryl cAMP) as well as that of the phosphodiesterase inhibitor IBMX. A major problem in studying the light-induced RPE responses with these pharmacological agents is that they may affect either or both the RPE and the neural retina—in this case, especially the photoreceptors. It was necessary, therefore, to separate any observed effects into their components. We used measurements of subretinal potassium concentration and the a-wave of the ERG to monitor photoreceptor function in the retina-RPE-choroid preparation. Effects thought to originate at the RPE could be confirmed, then, by studying the RPE-choroid isolated from the neural retina. Portions of this work have appeared in abstract form.15

Materials and Methods

All experiments were performed on white chicks (Gallus domesticus), 1 to 10 days old. All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. Complete methods are presented in the accompanying paper.16

Adenosine 3',5'-cyclic monophosphate (cAMP), dibutyryl cAMP (db-cAMP) or 3-isobutyl-1-methylxanthine (IBMX) was added to the retinal or choroidal perfusate. These chemicals were purchased from Sigma (St. Louis, MO).

For convenience, the following equations are repeated from the accompanying paper:16

\[ \Delta \text{TTP} = \Delta \text{TEP} + \Delta V_R \] (1)
\[ \Delta \text{TEP} = \Delta V_{ba} - \Delta V_{ap} \] (2)
\[ \Delta V_{ap} = \Delta V_{ap} \times \frac{R_{ba} + R_s}{R_{ap} + R_{ba} + R_s} \] (3)
\[ \Delta V_{ba} = \Delta V_{ap} \times \frac{R_{ba}}{R_{ap} + R_{ba} + R_s} \] (4)
\[ \Delta \text{TEP} = -[(R_0)/(R_{ap} + R_{ba} + R_s)] \Delta V_{ap} \] (5)

(\( \Delta V_{ap} \) is negative, so \( \Delta \text{TEP} \) is positive.)

\[ R_t = R_s \times \frac{R_{ap} + R_{ba}}{R_{ap} + R_{ba} + R_s} \] (6)

where TTP = trans-tissue potential; TEP = transepithelial potential; \( V_R \) = transretinal potential; \( V_{ap}(V_{ba}) \) = measured potential of the apical (basal) membrane of the RPE; \( V_{ap} \) = apical membrane battery; \( R_t \) = transepithelial resistance; \( R_{ap} \) (\( R_{ba} \)) = apical (basal) membrane resistance; \( R_s \) = paracellular shunt resistance. Throughout this paper, numerical results are given as mean values ± SEM.

Results

Cyclic AMP

Trans-tissue potential: As shown in Figure 1A (left), when the retina-RPE-choroid preparation was perfused on its retinal surface with 0.5 mM dibutyryl cyclic AMP, the dark-adapted trans-tissue potential first decreased and this was followed by a partial recovery of the potential towards baseline. Figure 1A also shows that the changes in trans-tissue potential originated mainly from the RPE since the changes in the transepithelial potential had essentially the same time-course and magnitude as those of the trans-tissue potential, while the transretinal potential changed little. For 17 tissues (cAMP 12, db-cAMP 5), the trans-tissue potential decreased an average maximum of 2.83 ± 0.06 mV from an average value of 6.15 ± 0.16 mV at about 6.5 min after switching perfusate. Upon returning to control perfusate these effects were reversed (Fig. 1A, right), with the trans-tissue potential recovering an average of 2.04 ± 0.09 mV in about 18 min. Since significant differences were not observed between effects of db-cAMP and cAMP, these data will be considered together.

To test the possibility that some of these RPE effects might be secondary to those on neural retinal cells, we studied the RPE-choroid preparation in the absence of the neural retina. Figure 1B shows that 0.5 mM cAMP added in the apical bath also decreased the transepithelial potential of the RPE-choroid preparation and the effect was reversible. This result suggested that these electrical effects of cAMP originated from direct effects on retinal pigment epithelial cells.

Choroidal application of 0.5 mM cAMP had effects that were essentially the same as retinal application, a decrease in the trans-tissue potential followed by partial recovery (Figs. 2, 3B). With 0.5 mM cAMP, the mean decrease in TTP was 0.91 ± 0.04 mV (n = 9; cAMP 4, db-cAMP 5), which was significantly smaller than with retinal application. Intraretinal recordings also indicated that these effects arose principally from the RPE and not from the neural retina and were reversed upon return to control perfusate (not shown).

Figure 2 shows the concentration dependence of effects on the trans-tissue potential of retinal (open circles) and choroidal (closed circles) cAMP. Application of the drug to either side of the tissue had a prominent effect at 10 µM with some effect observed even at 1.0 µM. Higher concentrations produced
Fig. 1. (A) Retina-RPE-choroid preparation. Effects of retinal perfusion with 0.5 mM db-cAMP on the trans-tissue potential and its transeptinal and transeptial components (left), and return to control perfusate (right). The trans-tissue potential was recorded differentially between the retinal and choroidal solutions. The transepithelial potential and transretinal potential were recorded simultaneously between a microelectrode placed in the subretinal space and respectively, the choroidal and retinal reference electrodes. Note that in this Figure and in Figures 5 and 11, the transretinal signal has been inverted, i.e., illustrated with the choroidal bath positive, so that it can be compared readily to the transepithelial potential. In this figure and in others the traces show “spike-like” c-wave responses produced by 4.0 sec flashes each 1.5 min. Note that the amplitude of these responses is not accurate because of digitization at this slow time base. (B) RPE-choroid preparation. Effects of apical perfusion with 0.5 mM cAMP on the transepithelial potential recorded differentially between the apical and choroidal solutions (left), and return to control perfusate (right). In both (A) and (B) the perfusate was changed to the test perfusate or returned to the control perfusate, as indicated at the bottom by the bars.

faster and larger decreases in the trans-tissue potential. Choroidal application produced smaller effects than retinal, with the difference between the size of the effects increasing as a function of concentration.

The transepithelial potential originates at both the apical and basal membranes of the RPE, representing the difference between the apical and basal membrane potentials. The decrease in transepithelial potential could result, therefore, from a hyperpolarization of the basal membrane, a depolarization of the apical membrane or both. To determine the locus of the effect on the RPE, intracellular recordings were obtained during retinal or choroidal cAMP perfusion. Figure 3A shows the apical and basal membrane potentials during retinal perfusion with 0.5 mM cAMP. Both membranes hyperpolarized with partial recovery, and the basal membrane hyperpolarization was larger, consistent with retinal cAMP hyperpolarizing the basal membrane with a passive hyperpolarization of the apical membrane due to shunting. Similar results were obtained in seven experiments (cAMP 5, db-cAMP 2). Figure 3B shows the result during choroidal perfusion with 0.5 mM cAMP. Again, both membranes hyperpolarized with partial recovery and the basal membrane hyperpolarization was larger, also consistent with the hyperpolarization originating at the basal membrane. Similar results were obtained in three experiments (cAMP 1, db-cAMP 2). These results indicated that cAMP in the choroidal bath had essentially the same effects as in the retinal bath, except for being smaller in size.

Resistance measurement: Retinal cAMP reversibly increased the trans-tissue resistance (Rtot) by an average of 12% (n = 14). Figure 4A shows that the time-course of the increase in Rtot was similar to that of the decrease in trans-tissue potential and resulted from
Fig. 3. Effects on RPE membrane potentials and the trans-tissue potential of (A) retinal perfusion and (B) choroidal perfusion with 0.5 mM cAMP. The trans-tissue potential was recorded as in Figure 1. Simultaneously, the apical membrane potential (V_{ap}) was recorded differentially between the intracellular microelectrode and the retinal perfusate, while the basal membrane potential (V_{ba}) was recorded between the microelectrode and the choroidal perfusate.

an increase in the RPE resistance (R_t) and not from a change in retinal resistance (R_R). This resistance increase could have originated from an increase in apical membrane (R_{ap}), basal membrane (R_{ba}) or paracellular shunt (R_s) resistance (eq. 6). Intracellular RPE recordings (Fig. 4B) revealed that retinal cAMP decreased the ratio of apical to basal membrane resistance, R_{ap}/R_{ba}, with a time-course similar to the decrease in trans-tissue potential and the increase in R_{tot}. The simplest explanation for these changes in
resistance parameters is an increase in basal membrane resistance. The results with choroidal cAMP were essentially the same except that the effect was smaller, with \( R_{\text{tot}} \) increasing an average of 7.8% (\( n = 6; \) cAMP 3, db-cAMP 3), and intracellular recordings also indicated a basal membrane origin for the effect (not shown).

**C-wave:** As shown in Figure 5A, c-waves elicited with a 4-sec flash at 1.5 min intervals became smaller with 0.5 mM retinal cAMP. There was also a decrease in the amplitude of the a-wave, and increase in b-wave amplitude as shown on an expanded time scale and with a higher gain in Figure 5B.

It is known that the c-wave is produced by a light-evoked decrease in subretinal \([K^+]_o\), and that the trans-tissue c-wave represents the sum of a negative-going potential generated in the neural retina (slow PIII) and a positive-going component from the RPE, the RPE c-wave. The decrease in c-wave amplitude could have resulted from changes in either or both of these components. We found that slow PIII decreased by an average of 14.4%, compared with a 35.5% reduction in the transepithelial c-wave (\( n = 9, 5 \) db-cAMP and 4 normal cAMP). To determine the origin of this c-wave decrease we measured the light-evoked decrease in subretinal \([K^+]_o\) using \( K^+ \)-selective microelectrodes. In six of nine experiments there was a clear reduction in the size of the light-evoked \([K^+]_o\) decrease by an average of 11.9%. (Fig. 5A illustrates a case in which the amplitude of the light-evoked \([K^+]_o\) decrease was not significantly altered by cAMP.) This effect was too small to be the sole cause of the decrease in the transepithelial c-wave (mean = 37.7% for these six experiments), but could account for most of the decrease in slow PIII (13.8%). (Note that during retinal perfusion with cyclic nucleotide the \([K^+]_o\) tended to decrease transiently, but this result was not fully documented.)

Figure 6A shows that the c-wave amplitude decrease and recovery paralleled in time-course the decrease and recovery of the trans-tissue potential. Figure 6B shows that the absolute magnitude of the c-wave decrease across all experiments (\( n = 20 \)) was positively correlated with the amplitude of the decrease in trans-tissue potential induced by retinal or choroidal cAMP, or db-cAMP. These data suggest that the mechanisms underlying the changes in the c-wave and trans-tissue potential were related.

---

**Fig. 5.** (A) Effects of retinal perfusion with 0.5 mM cAMP on the trans-tissue c-wave (ERG c-wave), and its transepithelial and tranretinal components. Also shown are light-evoked changes in subretinal potassium concentration \((V_{K^+})\) simultaneously recorded with a double-barreled \( K^+ \)-selective microelectrode in the subretinal space. Responses during retinal cAMP (middle) were recorded 20 min after changing the retinal perfusate to the test solution. Responses after cAMP (right) were recorded 30 min after returning to the control perfusate. C-waves were elicited by a 4.0 sec stimulus \((6 \times 10^{-5} \text{W/cm}^2)\). (B) Initial portions of the trans-tissue and tranretinal traces of (A) shown at a higher gain and with an expanded time scale in order to observe the a- and b-waves of the ERG. (C) Effects of retinal perfusion with 0.5 mM db-cAMP on the intracellularly recorded RPE c-wave membrane hyperpolarizations \((V_{ap} \) and \( V_{ba} \)), as correlated with changes in the trans-tissue c-wave. The recording configuration as in Figure 3. Stimulus as in (A).
Figure 5C shows selected basal and apical membrane hyperpolarizations in response to 4 sec flashes recorded before, during and after perfusion with 0.5 mM retinal db-cAMP. Also shown is the trans-tissue c-wave simultaneously recorded. db-Cyclic AMP increased both $\Delta V_{ap}$ and $\Delta V_{ba}$, with the increase in $\Delta V_{ba}$ being larger, such that a decrease in the trans-tissue c-wave was observed. These changes were reversed during the recovery phase, and similar results were observed in all five experiments. These responses are consistent with an increase in $R_{ba}$ during perfusion with cAMP causing a decrease in the c-wave (eqs. 3–5), and this conclusion is consistent with the changes in $R_{ap}/R_{ap}$ illustrated in Figure 4B. Parallel experiments with choroidal cAMP, including potassium and intracellular voltage measurements ($n = 3$, not shown), also indicated that the decrease in the RPE c-wave probably resulted from an increase in $R_{ba}$.

Light peak: As shown in Figure 7A, retinal cAMP reversibly decreased the amplitude of the light peak. Mean light peak amplitudes before, during and after retinal cAMP were $0.49 \pm 0.02$ mV, $0.14 \pm 0.02$ mV and $0.28 \pm 0.02$ mV respectively ($n = 8$; cAMP 4, db-cAMP 4). Recordings of the transepithelial potential and transretinal potential in Figure 7A show that the reduction of the light-peak amplitude resulted from a decline in the amplitude of the transepithelial light peak. No new potential was observed to develop across the neural retina that could have produced a reduction in the light peak.

We were unable to reduce light-peak amplitude significantly with choroidal application of 0.5 mM cyclic nucleotide (cAMP 3, db-cAMP 4), raising a question of whether the depression by retinal cAMP resulted from an effect on the neural retina and not on the RPE. However, a more likely hypothesis, was the overall lower responsiveness of the RPE to choroidal cAMP versus retinal cAMP (Figs. 2, 6B). Figure 7B shows the relationship between light-peak amplitude and the cAMP-induced decrease in trans-tissue potential at the time point of light peak measurement. Choroidal cAMP of 0.5 mM (closed circles) did not decrease the trans-tissue potential more than 1.0 mV, while 0.5 mM retinal cAMP always did. When we used a concentration of retinal cAMP, 10 $\mu$M (open triangles), that only decreased the trans-tissue potential by less than 1.0 mV, the light peak also was not affected, just as with 0.5 mM choroidal cAMP. These data suggested that the inability of choroidal cAMP to depress the light peak was due, at least in part, to the lower overall responsiveness of the RPE to choroidal cAMP. We have not, however, completely eliminated some additional unknown effect of retinal cAMP on the light-peak mechanism of the neural retina.
IBMX

Trans-tissue potential: As shown in Figure 8A, perfusion of the retina–RPE–choroid preparation on its retinal surface with 0.1 mM IBMX first decreased the trans-tissue potential and this was followed by an increase above the baseline level. For eight tissues, the average decrease and increase in trans-tissue potential as measured from the baseline were 0.53 ± 0.04 mV and 0.79 ± 0.05 mV, respectively. Figure 8A also shows that the changes in trans-tissue potential originated mainly from the RPE, with the changes in the transepithelial potential having a similar time-course to the changes in trans-tissue potential. There were also changes in the transretinal potential, however, that contributed significantly to the effect on the trans-tissue potential. Choroidal application of 0.1 mM IBMX had effects that were essentially the same as retinal application, transiently decreasing and then increasing the dark-adapted trans-tissue potential, 0.38 ± 0.06 mV and 1.02 ± 0.08 mV, respectively (n = 7). To determine if IBMX was directly affecting the RPE, we used the RPE–choroid preparation. Figure 8B shows that 0.1 mM IBMX added in the apical bath decreased the transepithelial potential of the RPE–choroid preparation and this was followed by a recovery or increase, but one that was smaller than in the retina–RPE–choroid preparation (Fig. 8A).

Measurements with K⁺-selective microelectrodes in the subretinal space showed that retinal (Fig. 9) or...
choroidal IBMX (not shown) first increased dark-adapted $[K^+]_o$ in the subretinal space and this was followed by a slow recovery. The concurrent recording of the trans-tissue potential in Figure 9 shows that its initial decrease and later increase paralleled in time-course the changes in subretinal $[K^+]_o$. These data and results to be presented below indicate that the effects of IBMX on photoreceptors led, via changes in subretinal $[K^+]_o$, to secondary effects on the RPE.

To determine the locus of the effect on the RPE, intracellular recordings were obtained during IBMX perfusion as shown in Figure 10 (0.1 mM IBMX). The changes in membrane potential occurred in three phases. In the first phase, the trans-tissue potential decreased as both membranes depolarized. The apical membrane depolarization was larger, consistent with the depolarization originating at the apical membrane. In the second phase, a small basal membrane hyperpolarization further decreased the trans-tissue potential. After these events, the trans-tissue potential increased (phase 3). This phase probably was due to a combined basal membrane depolarization and apical membrane hyperpolarization, which were difficult to resolve because of their opposite signs and small amplitudes. Essentially similar results were obtained when 0.5 mM IBMX was added to the choroidal bath.

C-wave: Retinal IBMX had a prominent effect on the c-wave (Fig. 11A), greatly increasing its amplitude, and increasing the amplitudes of the transepithelial and transretinal potentials that underlie it. Figure 11A (bottom) also shows that the light-evoked $K^+$ decrease, recorded simultaneously with a $K^+$-selective microelectrode in the subretinal space, also underwent a substantial increase in amplitude (see also Fig. 12). This would result from an increase in the light response of the photoreceptors. The photoreceptor response can be observed more directly in these recordings as the a-wave of the trans-tissue recording and as the leading edge of the response in the transretinal recording. As shown in Figure 11B both of these responses increased in size with retinal IBMX.

While it is clear that the principal reason for the increase in c-wave amplitude is the increase in the size of the light-evoked $[K^+]_o$ decrease, there also may have been changes in the resistance parameters of the RPE, as there were with cAMP perfusion. The RPE resistances were not assessed, however, because any small changes would have been masked by the effect on the c-wave of the large change in the light-evoked potassium response.

Figure 11C shows selected basal and apical c-wave membrane hyperpolarizations recorded before, during and after retinal perfusion with 0.1 mM IBMX.

- Fig. 9. Effect of retinal perfusion with 0.1 mM IBMX on subretinal potassium concentration ($V_K^+$) and the trans-tissue potential. In the in vitro chick preparation dark-adapted $[K^+]_o$ is approximately 5.4 mM, or 0.4 mM greater than in the control perfusate.23
- Fig. 10. Effects on RPE membrane potentials and the trans-tissue potential of retinal perfusion with 0.5 mM IBMX. The trans-tissue potential was recorded as in Figure 1, and membrane potentials as in Figure 3. In phase 1, the trans-tissue potential decreased and this was caused by a depolarization of $V_{ap}$. In phase 2, as the trans-tissue potential continued to decrease, $V_{ap}$ hyperpolarized. In phase 3, the trans-tissue potential increased and this appeared to be caused by a combined depolarization of $V_{ba}$ and hyperpolarization of $V_{ap}$.
Fig. 11. (A) Effects of retinal perfusion with 0.1 mM IBMX on the trans-tissue c-wave (ERG c-wave), and its transepithelial and transretinal components. Also shown are light-evoked changes in subretinal potassium concentration (\(V_{K^+}\)) simultaneously recorded. The recording configuration as in Figure 5A. Responses during retinal IBMX (middle) were recorded 20 min after changing the retinal perfusate to the test solution. Responses after IBMX (right) were recorded 30 min after returning to the control perfusate. C-waves were elicited by a 4.0 sec stimulus (6 \(\times 10^{-5}\) W/cm²). (B) Initial portions of the trans-tissue and transepithelial traces of (A) shown at a higher gain and with an expanded time scale in order to observe the a-wave of the ERG. Note that the rate of digitization of these records is too small to resolve rapid changes in the initial slope of the a-wave. (C) Effects of retinal perfusion with 0.1 mM IBMX on the intracellularly recorded RPE c-wave hyperpolarizations (\(V_{ap}\) and \(V_{ba}\)), as correlated with changes in the trans-tissue c-wave. The recording configuration as in Figure 3. Responses during retinal IBMX (middle) were recorded 15 min after changing the retinal perfusate to the test solution. Responses after IBMX (right) were recorded 15 min after returning to the control perfusate. Stimulus as in (A).

Also shown is the trans-tissue c-wave simultaneously recorded. As expected, retinal IBMX increased both membrane hyperpolarizations (\(V_{ap}\) and \(V_{ba}\)). Choroidal IBMX produced identical effects, suggesting that IBMX reached the photoreceptors during choroidal perfusion.

**Light peak**: As shown in Figure 12, retinal IBMX also strikingly increased the amplitude of the light peak and this effect was reversible. The mean light peak amplitudes before, during and after retinal IBMX were 0.60 ± 0.08 mV, 1.08 ± 0.12 mV and 0.58 ± 0.07 mV, respectively (n = 5). Choroidal IBMX also increased light-peak amplitude in the same manner as retinal IBMX. The mean light-peak amplitudes before, during and after choroidal IBMX were 0.68 ± 0.10 mV, 1.12 ± 0.29 mV and 0.56 ± 0.18 mV, respectively (n = 3).

**Discussion**

**cAMP**

We have found that perfusion of the chick retina-RPE-choroid on either its retinal or choroidal side with cAMP (or db-cAMP) hyperpolarized the RPE basal membrane and increased its resistance. Effects were observed with concentrations of cAMP as low as 1 \(\mu\)M and the effect on the trans-tissue potential increased in a dose-dependent manner up to the highest concentration tested (500 \(\mu\)M). Choroidal perfusion always produced smaller effects than retinal perfusion, most likely because of the diffusion barrier presented by the choroid, and perhaps because of the larger surface area of the apical membrane compared with the basolateral membrane. It is also possible that the RPE basolateral membrane takes up cyclic nucleotide less effectively than the apical membrane.

The most extensive previous studies of the effects of cAMP on RPE ionic mechanisms have been conducted on the isolated RPE-choroid of the bullfrog (R. catesbeiana). An increase in intracellular cAMP levels produced by a variety of methods has been shown to increase the secretion of NaCl and the absorption of K⁺ by modifying the active transport of these three ions, that is, an inhibition of active Cl⁻ transport and a stimulation of active Na⁺ and K⁺ transport.
Before | During retinal IBMX | After

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 12. Effects of retinal perfusion with 0.1 mM IBMX on the trans-tissue light peak (light peak of DC ERG). With a double-barreled K⁺-selective microelectrode in the subretinal space, the trans-epithelial and transretinal potentials as well as the light-evoked changes in subretinal potassium concentration (V,K⁺) were simultaneously recorded as in Figure 5A. Responses during retinal IBMX (middle) were recorded 60 min after changing the retinal perfusate to the test solution. Responses after retinal IBMX (right) were recorded 60 min after returning to the control perfusate. Light peaks were elicited by a 300 sec stimulus (6 × 10⁻⁵ W/cm²).

There is also a decrease in net fluid transport in the retina to choroid direction. cAMP in frog also has been found to produce a complex sequence of changes in RPE membrane potentials and apparent resistances, as well as a stimulation of the Na⁺-K⁺ pump on the apical membrane. The effect of cAMP on frog RPE membrane potentials and resistances was studied extensively recently using 1.0 mM cAMP plus 0.5 mM IBMX. The important point for our purposes is that the initial event, which was also the dominant response, was a depolarization of the basal membrane accompanied by a decrease in resistance. This was hypothesized to be produced by an increase in basal membrane anion conductance.

These results in frog contrast sharply with those in chick where opposite changes in membrane potential and resistance dominated the response, suggesting species differences in the response to changes in RPE cAMP levels, though differences in experimental conditions also could contribute to these results. Recently, we obtained evidence for a DIDS-inhibitable basal membrane Cl⁻ conductance in chick RPE. Since Cl⁻ is distributed above equilibrium across the chick RPE basal membrane, a decrease in Cl⁻ conductance would hyperpolarize the basal membrane and increase its resistance. Thus, the cAMP-induced hyperpolarization of the basal membrane may result from a decrease in basal membrane anion conductance. This could result from direct modulation of the conductance by cAMP, or by altering intracellular ion activities whereby the driving force on the conductance would be changed.

There is evidence from studies in fish and amphibians that exogenous cAMP can have direct effects on photoreceptors, where it acts as a second messenger in rod and cone retinomotor movements. While cAMP is present in the rods and cones of mammalian retinas, evidence for a specific role is not yet clear (for reviews see refs. 27 and 28). In the present experiments in chick we observed that exogenous cAMP produced a small (11.9%) reduction in the light-evoked decrease of subretinal [K⁺]₀ in six of nine experiments, and also decreased the amplitude of the ERG a-wave in some cases. Taken together these results suggest that exogenous cAMP can reduce the amplitude of the photoreceptor's response to light in chick, although the mechanism of this putative reduction in the photosresponse remains unknown.

Light peak: In the isolated, perfused eye of cat, Dawis and Niemeyer found that db-cAMP (480 μM) increased the standing potential and reduced light-peak amplitude. While intracellular recordings were not obtained, the most likely mechanism for this result, as they suggested, is a depolarization of the basal membrane accompanied by a decrease in basal membrane resistance. This is opposite to the effects of cAMP in chick, that is, a decrease in transepithelial potential, hyperpolarization of the basal membrane and increase in basal membrane resistance. That cAMP suppresses the light peak in chick and cat cannot be taken, necessarily, as evidence for a role in the light-peak mechanism. In cat, the depressed light peaks were elicited following a large cAMP induced increase in standing potential, and in chick the degree of light-peak suppression was related to the size of the decrease in trans-tissue potential that followed perfusion with retinal or choroidal cAMP. These results agree with our previous observations, summarized in the accompanying paper, that for both increases and decreases in standing potential (or trans-tissue potential) the degree of light-peak suppression is a function of the size of the change in standing potential.
tial, and that this effect is relatively nonspecific since it can be produced by any of a variety of pharmacological agents and experimental manipulations.

**IBMX**

The effects of IBMX were more complex than those of cAMP, since they involved, as expected, substantial changes in photoreceptor activity, which also altered subretinal $\left[ K^+ \right]_o$. Thus, the presumed direct effects on intracellular RPE cAMP levels were masked by these photoreceptor-dependent changes. The direct effects could be observed in the RPE–choroid preparation, where IBMX produced a decrease in the transepithelial potential (Fig. 8B), and this closely resembled the decrease produced in a similar preparation by perfusion with cAMP (Fig. 1B).

In the retina–RPE–choroid preparation, however, the initial decrease in transepithelial potential was much smaller with IBMX than with cAMP (compare Figs. 1A and 8A), and the dominant effect of IBMX was a subsequent increase in the transepithelial potential. In addition, the transepotential also exhibited a small decrease followed by a larger slow increase (Fig. 8A). Recordings of subretinal $\left[ K^+ \right]_o$ showed clearly that the changes in intracellular potential, the initial decrease followed by the larger increase, paralleled the time-course of $\left[ K^+ \right]_o$ during IBMX perfusion (Fig. 9). Thus, the initial decrease in transepithelial potential that resulted from the decrease in transepithelial potential paralleled an initial increase in $\left[ K^+ \right]_o$, while the subsequent increase in these potentials was concordant with a slower decrease in $\left[ K^+ \right]_o$.

The effects of IBMX most likely originated from the reduced hydrolysis of cGMP in the photoreceptor outer segment that followed the inhibition of the phosphodiesterase. This increases the conductance of the light-sensitive channels of the outer segment and depolarizes the photoreceptors. In the dark-adapted state, the result is an increase in the dark current, which is inward at the outer segment and carried mostly by Na+, and outward at the inner segment and carried by K+. Increasing the dark current with IBMX then, would increase the efflux of K+ at the inner segment, causing the observed initial increase in $\left[ K^+ \right]_o$. Similar effects on subretinal $\left[ K^+ \right]_o$ were first observed by Oakley studying the effects of low $\left[ Ca^{2+} \right]_o$ perfusion of the isolated toad retina, since low $\left[ Ca^{2+} \right]_o$ is also known to increase the light-sensitive conductance. He also noted the transient nature of the increase in subretinal $\left[ K^+ \right]_o$ and attributed the recovery of $\left[ K^+ \right]_o$ stimulation of the photoreceptor Na+–K+ pump, a result of the increase in photoreceptor intracellular Na+ concentration.

Assuming that the changes in $\left[ K^+ \right]_o$ during perfusion with IBMX originated at the photoreceptor, they should contribute to the observed changes in transepithelial potential. In this case the initial increase in $\left[ K^+ \right]_o$ would depolarize the apical membrane and the subsequent increase should hyperpolarize it. Similar effects should occur at the portion of the Müller cell membrane that adjoins the subretinal space. These effects were confirmed for the RPE in intracellular recordings (Fig. 11), by the observation of an initial apical depolarization during phase 1 and later hyperpolarization during phase 3. Added in, as well, were basal membrane events, presumably due to an increase in intracellular levels of cAMP in the RPE (eg, the initial basal membrane hyperpolarization in phase 2). It is clear from such intracellular recordings that the effects that can be explained as RPE responses to the changes in subretinal $\left[ K^+ \right]_o$ masked any direct effects of IBMX on intracellular levels of cAMP in the RPE.

Dawis and Niemeyer used theophylline to inhibit phosphodiesterase in the isolated perfused cat eye. Theophylline had been found to be much less effective than IBMX in altering the light response of photoreceptors in toad (see below) and Drugs perfused through the circulation should rapidly reach the RPE through the choriocapillaries. Dawis and Niemeyer reasoned, therefore, that theophylline might more strongly affect the RPE than photoreceptors, to increase intracellular levels of cyclic nucleotide. As discussed above, they had earlier shown that db-cAMP perfusion increased the standing potential of the perfused cat eye. Theophylline produced a prominent decrease in standing potential, which was followed by an increase. Based on the findings presented here in chick, this decrease in standing potential could result from an apical membrane depolarization subsequent to an increase in subretinal $\left[ K^+ \right]_o$, which would be evidence for a substantial effect on the photoreceptors. Other evidence of theirs did not support an effect upon photoreceptors. Alternatively, the decrease in standing potential could originate from a basal membrane hyperpolarization, a consequence of an increase in RPE cAMP levels, which would be, in its polarity, consistent with out findings with cAMP in chick. Their observation of a substantial decrease in c-wave amplitudes during theophylline perfusion is also consistent with a hyperpolarization of the basal membrane. (Left unexplained is why db-cAMP perfusion leads only to an increase in standing potential.)

It is well known that IBMX also increases the amplitude of the light response of photoreceptors and This explains the increase in a-wave amplitude observed in the current experiments (Fig. 11B), as well as the larger size of the light-evoked subretinal
changes in \([K^+)_o\) (Figs. 11A, 12). Oakley\(^3\) also had shown earlier that low \([Ca^{2+})_o\) perfusion in toad retina increased the amplitude of light-evoked \([K^+)_o\) responses. He also reported a faster initial slope for the light-evoked \([K^+)_o\) decrease in low \([Ca^{2+})_o\), and this was also found for the chick with IBMX (compare light-evoked \([K^+)_o\) responses in Fig. 11A).

Two light-evoked ERG responses of the RPE depend directly on the light-evoked changes in subretinal \([K^+)_o\), the c-wave and the fast oscillation. The increase in the \([K^+)_o\) response to light, therefore, increased the RPE component of the c-wave, as well as slow PIII, the Müller cell component of opposite polarity (Figs. 11A, 12). The fast-oscillation trough can be identified as the negative trough in potential that followed the c-wave in Figure 12. It originates from a delayed hyperpolarization of the RPE basal membrane.\(^3\)\(^3\)\(^4\) As expected, it also was considerably larger in amplitude during IBMX perfusion (Fig. 12). The light peak is thought not to depend directly on the size of the light-evoked decrease in subretinal \([K^+)_o\),\(^3\)\(^5\) but it may well depend upon the size of the photoreceptor’s light response. The striking increase in light-peak amplitude with IBMX suggests, therefore, that the quantity of the putative light-peak substance may vary directly with the size of the photoresponse.

Key words: cAMP, IBMX, chick, retina, subretinal space, RPE, electroretinogram

References


