cAMP Inhibits Transepithelial Chloride Secretion across Bovine Ciliary Body/Epithelium

Chi-Wai Do,1,2 Chi-Wing Kong,1 and Chi-Ho To1

PURPOSE. To investigate the potential significance of cAMP in the regulation of Cl− transport across the bovine ciliary body/epithelium (CBE).

METHODS. Fresh native bovine CBE preparation was mounted in a modified Ussing chamber. The effects of cAMP-stimulating agents on short-circuit current (Isc) and net 36Cl− secretion were determined.

RESULTS. Addition of cAMP-stimulating agents inhibited net Cl− secretion. Forskolin, when added bilaterally, reduced Cl− secretion by 60%. Similarly, bilateral isoproterenol or vasoactive intestinal peptide inhibited Cl− transport by 15% and 37%, respectively, suggesting a cAMP-sensitive Cl− transport across the ciliary epithelium. This notion was supported by the exogenous application of 8-bromo-cAMP (8-Br-cAMP) or 3-isobutyl-1-methylxanthine (IBMX), which reduced the net Cl− secretion by 49% and 85%, respectively. In unstimulated preparations, addition of 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) to the blood side did not have effects on Isc and net Cl− transport, indicating that Cl− reabsorption was negligible under baseline conditions. Also, pretreatment with NPPB from the blood side did not prevent forskolin-induced Isc inhibition, suggesting that the inhibition of Cl− transport did not result from the facilitation of Cl− reabsorption. However, pretreatment with heptanol from both sides completely blocked the forskolin-induced Isc inhibition, suggesting that cAMP may reduce Cl− transport by uncoupling the intercellular gap junctions.

CONCLUSIONS. The results suggest that cAMP plays a crucial role in modulating Cl− secretion across the ciliary epithelium. The effect is possibly mediated, at least in part, by the regulation of the permeability of gap junctions between pigmented and nonpigmented ciliary epithelial cells. (Invest Ophthalmol Vis Sci. 2004;45:3638–3643) DOI:10.1167/iovs.03-1343

Glaucoma occurs when intraocular pressure (IOP) elevates to a level at which the optic nerve fibers are damaged. It is known that IOP is not constant and displays diurnal variation. This is observed both in normal and in glaucomatous subjects, although the latter demonstrate a larger fluctuation. The circadian rhythm suggests that the secretion of aqueous humor is precisely controlled, although a clear understanding of the physiological mechanisms for the regulation remains elusive.

Many signaling pathways may contribute to the regulation of the formation of aqueous humor. Adenosine 3′,5′-cyclic monophosphate (cAMP) is an important second messenger responsible for the regulation of aqueous humor formation and IOP. Agents such as isoproterenol (a β-adrenergic agonist), forskolin (a direct activator of adenylate cyclase), and vasoactive intestinal peptide (VIP) have been shown to stimulate cAMP formation in the ciliary processes of human and rabbit. These cAMP-stimulating agents exert considerable effects on the formation of aqueous humor, and thereby on IOP, in both experimental animals and humans. For example, isoproterenol significantly stimulates formation of aqueous humor when injected into the anterior chamber of monkey eyes. Similarly, administration of VIP either intracamerally or intravenously increases aqueous humor formation in the same species. In addition, it has been shown that forskolin and cAMP enhance the regulatory volume decrease (RVD) and trigger shrinkage of human nonpigmented epithelial (NPE) cells under isosmotic conditions. In isolated dog NPE cells, forskolin and cAMP induce a Cl− current along with a concomitant decrease in cell volume. These findings suggest that stimulation of cAMP formation facilitates ion transport to the posterior chamber, resulting in an increased rate of formation of aqueous humor. On the contrary, it has been demonstrated that topical isoproterenol decreases the formation of aqueous humor and IOP in human. Topical and intravitreal administration of forskolin or VIP reduce aqueous humor formation and IOP among different species, including rabbit, monkey, and human. Moreover, forskolin and isoproterenol have been shown not only to reduce the formation of aqueous humor and IOP, but also to increase the cAMP concentration in aqueous humor. These opposing findings cast considerable doubt on the precise role of cAMP in regulating the formation of aqueous humor.

Transepithelial Cl− secretion across the ciliary epithelium probably plays an important role in the formation of aqueous humor among different species, including ox17–19 and rabbit.20,21 In the present study, we investigated the effects mediated by cAMP on both electrical parameters and Cl− transport. This information may be useful in understanding the mechanisms that regulate the formation of aqueous humor.

METHODS

The procedures for tissue preparation, electrical measurements, and determination of Cl− flux have been described previously. In brief, a sector of ciliary body/epithelium (CBE) was isolated from freshly enucleated bovine eyes. The excised CBE preparation was mounted in a modified, continuously perfused chamber with a cross-sectional area of 0.30 cm2 in the chamber cavity (Fig. 1).

Fluid perfusion through the chamber cavity was driven by a microprocessor-controlled syringe infusion pump (Cole-Palmer Instrument, Ltd., Chicago, IL). Ringer solution was delivered to both sides of the preparation at a rate of 10 mL·h−1. The temperature of the solution was maintained at 37°C. A dual-voltage clamp unit (DVC-1000: World Precision Instruments, Sarasota, FL) was used to monitor the electrical

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parameters of the preparation. The potential difference (PD) across the preparation was recorded with a pair of Ag/AgCl cartridge electrodes (EKV; World Precision Instruments) filled with 154 mM NaCl polyacrylamide gel. Before mounting the preparation, the blank resistance of the chamber (in the absence of tissue) was measured and compensated. Then, the preparation was mounted and monitored for measurement of the electrical parameters. A pharmacological agent was added to the chamber only when PD was stable for at least 15 minutes. Thereafter, the preparation was allowed to equilibrate until the full effect of the agent on the electrical parameters was shown. A simultaneous plot of Isc was used to monitor the viability of the preparation. The unidirectional \( { }^{36}\text{Cl}^- \) fluxes were determined under short-circuited conditions, which effectively eliminate the electrical driving force of passive diffusion, while maintaining the primary active ion transport.\(^2\) To minimize the variability between individual eyes, only those data from preparations of the same eye were compared. This was achieved by mounting two preparations from the same eye: one preparation was used for the measurement of influx (J\(_{ba}\): blood-to-aqueous), and the other preparation was mounted in another chamber for backflux measurement (J\(_{ab}\): aqueous-to-blood). The net flux was calculated in terms of the difference between J\(_{ba}\) and J\(_{ab}\). In other words, a single net flux data point was obtained from each eye. For the flux measurements, perfusates from either side of the preparation were collected separately at 12-minute intervals; at least three to four consecutive samples were taken. The radioactivity of the samples was measured with a liquid scintillation counter (Wallac 1414 Winspectral DSA; Wallac, Helsinki, Finland) after being mixed with 15 mL of scintillation cocktail (NBCS104; Amersham Radiochemicals, UK). The mean flux was calculated from the average value of the consecutive samples, and its value was expressed in microequivalents per hour per square centimeter (\(\mu\text{Eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}\)).

All the solutions were freshly prepared. The control HCO\(_3^-\)-containing Ringer solution composed of (in mM) NaCl 113.0, KCl 4.6, NaHCO\(_3\) 21.0, MgSO\(_4\) 0.6, D-glucose 7.5, glutathione (reduced form) 1.0, Na\(_2\)HPO\(_4\) 1.0, HEPES 10.0, and CaCl\(_2\) 1.4. The solution was bubbled with 95% O\(_2\)-5% CO\(_2\) for 15 minutes, and the pH was adjusted to

**Table 1. Baseline Unidirectional \( { }^{36}\text{Cl}^- \) Fluxes Measured in Paired Preparations under Short-Circuited Conditions, Using Two Sets of Chambers, S\(_1\) or S\(_2\)**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>J(_{ba})</th>
<th>I(_{sc})</th>
<th>R(_t)</th>
<th>J(_{ab})</th>
<th>I(_{sc})</th>
<th>R(_t)</th>
<th>J(_{net})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(_1)</td>
<td>14</td>
<td>4.74 ± 0.26</td>
<td>-8.3 ± 1.3</td>
<td>72 ± 5</td>
<td>3.71 ± 0.17</td>
<td>-7.7 ± 0.7</td>
<td>71 ± 4</td>
<td>1.03 ± 0.28</td>
</tr>
<tr>
<td>S(_2)</td>
<td>8</td>
<td>2.98 ± 0.28</td>
<td>-9.7 ± 1.4</td>
<td>113 ± 12</td>
<td>1.91 ± 0.14</td>
<td>-7.6 ± 0.7</td>
<td>127 ± 10</td>
<td>1.06 ± 0.32</td>
</tr>
</tbody>
</table>

Flux (J), tissue resistance (R\(_t\)) and short-circuit current (I\(_{sc}\)) values are expressed as \(\mu\text{Eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}, \Omega \cdot \text{cm}^2\), and \(\mu\text{A} \cdot \text{cm}^{-2}\), respectively. Two preparations from the same eye were mounted in two identical chambers of either S\(_1\) or S\(_2\) for the simultaneous determination of J\(_{ba}\) and J\(_{ab}\). Data are expressed as the mean ± SEM.

* Significantly different from zero (Student’s paired t-test, \(P < 0.05\)).

**FIGURE 1.** Configuration of the modified Ussing chamber (S\(_2\)). (a) Front view, (b) lateral view, (c) top view. Two different sets of chambers (S\(_1\) or S\(_2\)) were used for the experiments. Their configurations were similar, except that in S\(_2\), a sliding platform was introduced, which considerably reduced the lateral movement between two half chambers during tissue clamping. This maneuver significantly minimized the tissue damage caused by mounting the preparation.
TABLE 3. Effects of 10 μM Forskolin (FSK), 10 μM Isoproterenol (ISO), and 0.1 μM VIP on Cl⁻ Transport

<table>
<thead>
<tr>
<th></th>
<th>Blood-to-Aqueous</th>
<th>Aqueous-to-Blood</th>
<th>Net Flux</th>
<th>Flux Inhibition (%)</th>
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<tbody>
<tr>
<td></td>
<td>J_{ba}</td>
<td>I_{ac}</td>
<td>R_{t}</td>
<td>J_{ab}</td>
</tr>
<tr>
<td>FSK*</td>
<td>6</td>
<td>4.49 ± 0.37</td>
<td>-6.6 ± 0.8</td>
<td>78 ± 7</td>
</tr>
<tr>
<td>ISO*</td>
<td>7</td>
<td>4.77 ± 0.34</td>
<td>-7.3 ± 1.7</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>VIP†</td>
<td>7</td>
<td>3.59 ± 0.24</td>
<td>-11.8 ± 2.2</td>
<td>101 ± 9</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM. The baseline net Cl⁻ influx of 1.03 μEq · h⁻¹ · cm⁻² found in S₁ and 1.06 μEq · h⁻¹ · cm⁻² found in S₂ were used as a reference for comparison to other experimental conditions, using the same set of chambers. Flux (J), tissue resistance (Rₜ), and I_{ac} are expressed as μEq · h⁻¹ · cm⁻², Ω · cm², and μA · cm⁻², respectively.

* Measured using S₁.
† Measured using S₂.

7.4 before use. Then, the solution was loaded into the syringes immediately so that the desired concentration of HCO₃⁻ could be maintained. Isoproterenol, forskolin, 1,9-dideoxyforskolin, VIP, 8-bromo-cAMP (8-Br-cAMP), bumetanide, DIDS (4,4'-disothiocyanostilbene-2,2'-disulfonylic acid), and IBMX (3-isobutyl-1-methylxanthine) were purchased from Sigma-Aldrich (St. Louis, MO), and NPPB (5-nitro-2-(3-phenylpropylamino)-benzoic acid) was obtained from Biomol Research (Plymouth Meeting, PA). Hepanol was purchased from Fluka Chemie (Buchs, Switzerland), and Na³⁶Cl in aqueous solution was obtained from Amersham (Arlington Heights, IL). The ³⁶Cl⁻ activity in Ringer solution was adjusted to 0.25 μCi · mL⁻¹. Some chemicals were dissolved in dimethyl sulfoxide (DMSO) before they were added to the Ringer solution. The final concentration of DMSO in the Ringer solution was adjusted to less than 0.1%, the level at which it was shown not to affect either electrical parameters or Cl⁻ transport.

RESULTS

Under baseline conditions, the unidirectional blood-to-aqueous flux (J_{ba}) was significantly larger than the paired aqueous-to-blood flux (J_{ab}), with an average net Cl⁻ secretion of approximately 1.04 μEq · h⁻¹ · cm⁻². However, the I_{sc} of the paired preparations were not significantly different as shown in Table 1, the measurements of unidirectional and net Cl⁻ fluxes were determined with two different sets of chambers (S₁ or S₂) under short-circuited conditions. The measurement of basal Cl⁻ secretion with S₁ has been reported, and it is currently used as a reference for comparison to other experimental conditions. Introducing the sliding platform in S₂ effectively minimized the edge damage caused by tissue clamping (Fig. 1), resulting in reduced leakage, which was reflected in the significant reduction of unidirectional Cl⁻ fluxes in both directions compared with S₁. However, the net Cl⁻ secretion was unaffected.

The effects of cAMP-stimulating agents on transepithelial Cl⁻ transport are summarized in Table 2. Forskolin (10 μM) significantly inhibited the Cl⁻ transport by 60% when added to both sides of the preparation. Bilateral isoproterenol (10 μM) and VIP (0.1 μM) also decreased the net Cl⁻ transport by 15% and 37%, respectively. In contrast, 1,9-dideoxyforskolin (10 μM), a forskolin analogue that does not stimulate CAMP formation, had no effect on I_{sc} when applied bilaterally (data not shown). To study whether CAMP inhibits Cl⁻ transport in ciliary epithelium, we examined the effects of 8-Br-cAMP (a membrane-permeable cAMP analogue) and IBMX (a phosphodiesterase inhibitor) on I_{sc} and Cl⁻ transport. Exogenous application of 8-Br-cAMP (0.1 mM) bilaterally reduced the I_{sc} by 10% ± 4% (n = 13) and IBMX also inhibited the I_{sc} by 36% ± 4% (n = 16) when added to both sides of the preparation. In agreement with these findings, bilateral application of 8-Br-cAMP inhibited Cl⁻ secretion by approximately 50% (Table 3). Similarly, IBMX significantly reduced Cl⁻ transport by 85% when added to both sides of the preparation.

To investigate whether the mechanism by which cAMP inhibits net Cl⁻ secretion was by enhancing the unidirectional Cl⁻ transport from the posterior chamber to the blood plasma (Cl⁻ reabsorption), we studied the effects on I_{sc} of the Cl⁻ channel blockers NPPB and DIDS on the blood side as well as bumetanide on the aqueous side. Under baseline conditions, none of these transport inhibitors stimulated the I_{sc} (Table 4). The lack of Cl⁻ reabsorption under baseline conditions was further supported by two additional experiments in which NPPB on the blood side had no effect on the net Cl⁻ secretion (1.02 ± 0.05 μEq · h⁻¹ · cm⁻²) across the bovine CBE. In addition, pretreatment with NPPB from the blood side did not affect forskolin-induced I_{sc} inhibition (Fig. 2), suggesting that the inhibition of net Cl⁻ secretion by cAMP was not due to the facilitation of Cl⁻ reabsorption.

It is possible that cAMP may have inhibited net Cl⁻ secretion by regulating the permeability of the gap junctions in the ciliary epithelium. Figure 3 shows the sequential effects of heptanol (a commonly used gap junction inhibitor) and ouabain on electrical measurements. Addition of 3.5 mM heptanol to both sides produced a significant I_{sc} inhibition of 60% to 80%. Pretreatment with heptanol, however, did not affect the biphasic responses of ouabain on I_{sc}. In the presence of heptanol, ouabain triggered a rapid stimulation of I_{sc} (approximately three times larger than the baseline I_{sc}), followed by a gradual and complete inhibition of I_{sc}. Pretreatment with heptanol, however, completely prevented the forskolin-induced I_{sc} inhibition.

TABLE 3. Effects of 0.1 mM 8-Br-cAMP (cAMP) and 1 mM IBMX on Cl⁻ Transport

<table>
<thead>
<tr>
<th></th>
<th>Blood-to-Aqueous</th>
<th>Aqueous-to-Blood</th>
<th>Net Flux</th>
<th>Flux Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J_{ba}</td>
<td>I_{ac}</td>
<td>R_{t}</td>
<td>J_{ab}</td>
</tr>
<tr>
<td>cAMP*</td>
<td>7</td>
<td>3.02 ± 0.13</td>
<td>-6.2 ± 1.1</td>
<td>158 ± 10</td>
</tr>
<tr>
<td>IBMX†</td>
<td>7</td>
<td>2.25 ± 1.1</td>
<td>-3.7 ± 0.6</td>
<td>142 ± 7</td>
</tr>
</tbody>
</table>

Baseline net Cl⁻ influx of 1.06 μEq · h⁻¹ · cm⁻² found in S₂ was used as a reference for comparison to other experimental conditions. Flux (J), tissue resistance (Rₜ), and I_{ac} are in μEq · h⁻¹ · cm⁻², Ω · cm², and μA · cm⁻², respectively. The data are expressed as the mean ± SEM.

* Measured using S₁.
† Measured using S₂.
inhibition (Fig. 4), suggesting that CAMP may reduce the Cl⁻ transport by partially uncoupling the intercellular gap junctions between PE and NPE cells.

**DISCUSSION**

The physiological regulation of aqueous humor dynamics in vivo is complex. A single agent may bind to specific receptors in several distinct cell types, and each of them may influence aqueous humor dynamics in a different manner by stimulating or inhibiting the inflow, outflow, or both.⁵,⁶ Much effort has been devoted to investigation of the signaling cascades that may influence the formation of aqueous humor; however, the result is still inconclusive. Structural complexity of the ciliary epithelium as well as variations in transport components among studied species may account, at least in part, for the discrepancies. The use of the Ussing chamber technique in studying aqueous humor formation eliminates possible influences from the outflow pathway and also allows measurement of the electrical responses in the absence of systemic vascular and hormonal influences. In addition, the possible artifacts produced by the iris are excluded, because the cell body is exposed to the bathing solution.

Stimulation of CAMP production is observed with isoproterenol, forskolin, or VIP in both rabbit and human ciliary processes.⁴,⁵ It has been demonstrated that these CAMP-stimulating agents reduce the formation of aqueous humor and IOP⁴,⁵,⁶,¹⁰–¹² whereas other studies have found the opposite effects.⁶,⁷ In the present study, we found that these agents reduced the transepithelial Cl⁻ transport, suggesting a CAMP-sensitive Cl⁻ secretion across the ciliary epithelium. Forskolin was shown to be more effective in reducing Cl⁻ transport than VIP or isoproterenol. This was consistent with other studies in which forskolin elicited a larger stimulation of CAMP formation than VIP or isoproterenol.⁴,⁵ This was also supported by the experiments in which 1,9-dideoxyforskolin, a forskolin analogue that did not stimulate adenylate cyclase and thereby CAMP formation had no effect on Iᵥ. These findings imply that the inhibition of Cl⁻ transport may be due to the stimulation of CAMP formation. The notion of CAMP-sensitive Cl⁻ transport was strengthened by the experiments in which 8-Br-CAMP or IBMX caused a significant inhibition of both electrical parameters and net Cl⁻ secretion, supporting an inhibitory effect of CAMP on Cl⁻ transport across the bovine CBE. It also indicates that bovine CBE is a good model for humans, since CAMP or CAMP-stimulating agents can reduce the formation of aqueous humor in both cases. Our result was consistent with the finding that terbutaline, a β-adrenergic agonist, decreases the rate of formation of aqueous humor in the arterially perfused bovine eye.²⁵ It has been shown that the administration of either β-adrenergic agonists or antagonists lowers IOP by reducing the rate of aqueous secretion.¹²,²⁶ Timolol, a β-adrenergic antagonist, has been commonly used in glaucoma treatment to lower IOP by reducing the rate of formation of aqueous humor. However, the precise mechanisms of the action are not known. It has been shown that timolol reduces endogenous CAMP formation; however, whether the hypotensive effects are primarily mediated by the effects on CAMP remains unclear.²⁷,²⁸ Given that CAMP inhibits net Cl⁻ secretion across the bovine CBE, our results are consistent with the recent findings that timolol may act through CAMP-independent pathways in mediating its hypotensive effect.²⁷ The profound inhibitory effect of IBMX on Cl⁻ secretion (~85%) may suggest the involvement of other signaling cascades in the regulation of Cl⁻ transport. Apart from the stimulation of CAMP formation, blockade of phosphodiesterase with IBMX elicited a simultaneous stimulation of intracellular cGMP production, which may provide an alternative pathway for the regulation of aqueous humor secretion (unpublished observation). Taken together, these results suggest that CAMP is a key second messenger in the regulation of Cl⁻ secretion across the bovine CBE.

It has been shown that CAMP activates the Na⁺,K⁺,2Cl⁻ cotransporter in PE cells²¹ and stimulates Cl⁻ efflux in NPE cells.²⁹,³⁰ Presumably if CAMP activates the Na⁺,K⁺,2Cl⁻ cotransporter in PE cells and/or Cl⁻ channels in NPE cells, a stimulation of Iᵥ and Cl⁻ transport would be expected. In contrast, we have observed an inhibition of Iᵥ and Cl⁻ secretion by CAMP, suggesting that CAMP may play a minor role, if any, in the activation of Na⁺,K⁺,2Cl⁻ cotransporter in PE cells and/or Cl⁻ channels in NPE cells in this bovine preparation. Our finding was consistent with a study of isolated bovine NPE cells, where Cl⁻ conductance was unaffected by adrenergic stimulation, although the intracellular CAMP formation was increased.³¹ This result was different from a recent study of isolated rabbit ciliary epithelial bilayer in which isoproterenol increased the net blood-to-aqueous Cl⁻ flux.³² The exact reason for the discrepancy is unclear, but species difference may contribute to the observed differences.

The net Cl⁻ secretion can be inhibited by increasing unidirectional Cl⁻ reabsorption. Adenylate cyclase has been more abundantly found in NPE cells than in PE cells.³² Administration of VIP elicits a larger stimulation of intracellular CAMP production in NPE than in PE cells.³³ Additional evidence for the presence of Na⁺,K⁺,2Cl⁻ cotransporter in NPE cells³⁴ and Cl⁻ channels in PE cells³⁵,³⁶ has been described. The Na⁺, K⁺, 2Cl⁻ cotransporter in NPE cells has been shown to be stimulated by either β-adrenergic agonist³⁷ or forskolin.³⁸ Most recently, it has been demonstrated that CAMP stimulates whole-cell Cl⁻ currents and activates maxi-Cl⁻ channels in cultured and native bovine PE cells.³⁹,⁴⁰ Therefore, it is likely that CAMP reduces the net Cl⁻ transport by increasing the Cl⁻ reabsorp-

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**Table 4. Effects of 0.1 mM NPPB on Iᵥ and ΔIₛₑ of isolated rabbit ciliary epithelial bilayer.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control</th>
<th>Drug-Treated</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMTₐq₀</td>
<td>7±0.7</td>
<td>-3.0±0.6</td>
<td>-1.9±0.3</td>
</tr>
<tr>
<td>DIDSₐq₀</td>
<td>3±0.9</td>
<td>-6.2±0.6</td>
<td>-0.3±0.5</td>
</tr>
<tr>
<td>NPPBₐq₀</td>
<td>5±0.7</td>
<td>-4.2±0.7</td>
<td>-4.1±0.6</td>
</tr>
</tbody>
</table>

Data are expressed as μA · cm⁻² (mean ± SEM).

* Significantly different from control (Student’s paired t-test, P < 0.05).

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**Figure 2.** Sequential effects of 0.1 mM NPPB on the blood side (bl) and 10 μM forskolin (FSK) on both sides on Iᵥ across the isolated bovine CBE. The results were normalized and are expressed as the mean ± SEM, indicated by the error bars (n = 5). *Significantly different from control (repeated-measures ANOVA, P < 0.05).
tion through the activation of Na\(^+\), K\(^+\), 2Cl\(^-\) cotransporter in NPE cells and/or Cl\(^-\) channels in PE cells. Addition of bumetanide to the aqueous side or of Cl\(^-\) channels blockers to the blood side presumably decreases Cl\(^-\) uptake from the posterior chamber by NPE cells or Cl\(^-\) efflux into the ciliary stroma by PE cells. This may lead to the reduction of unidirectional Cl\(^-\) reabsorption, as reflected by an increase of \(I_{\text{sc}}\) and net Cl\(^-\) secretion. However, none of these transport inhibitors stimulated the \(I_{\text{sc}}\) under unstimulated conditions. In contrast, bumetanide reduced the \(I_{\text{sc}}\) by approximately 40%, which may reflect a decreased Cl\(^-\) uptake into the NPE cells and eventually reduce the net Cl\(^-\) secretion into the posterior chamber. Similarly, addition of NPPB to the blood side had no effect on net Cl\(^-\) secretion, suggesting the lack of Cl\(^-\) reabsorption under baseline conditions. Although NPPB from the blood side did not affect the baseline \(I_{\text{sc}}\), subsequent addition of forskolin caused a significant inhibition of \(I_{\text{sc}}\), suggesting that the inhibition of Cl\(^-\) transport with cAMP does not result solely from the facilitation of Cl\(^-\) reabsorption.35

It is plausible that cAMP reduces Cl\(^-\) secretion across the CBE by uncoupling the intercellular gap junctions between PE and NPE cells. As in other studies of the ciliary body preparations,18,35 addition of heptanol to both sides produced a significant inhibition of \(I_{\text{sc}}\) by 60% to 80%. Pretreatment with heptanol, however, did not affect the biphasic effect of ouabain on \(I_{\text{sc}}\). In the presence of heptanol, ouabain triggered a rapid stimulation of \(I_{\text{sc}}\) followed by a gradual and complete inhibition of \(I_{\text{sc}}\). This experiment’s result is important in two aspects: It suggests that (1) the Na\(^+\), K\(^+\)-activated adenosine triphosphatase (ATPase) remains functionally intact after heptanol treatment and that (2) when the ion transfer between PE and NPE cells is blocked by heptanol, inhibition of other ion transport elements could still be detected by \(I_{\text{sc}}\). To investigate whether cAMP acts on the gap junctions in ciliary epithelium, we studied the sequential effect of heptanol and forskolin on \(I_{\text{sc}}\). A significant inhibition of \(I_{\text{sc}}\) was observed (~60%) when heptanol was added to the preparation bilaterally. Subsequent addition of forskolin to these preparations produced no further inhibition of \(I_{\text{sc}}\), suggesting an uncoupling effect of cAMP on intercellular gap junctions. It is possible that the uncoupling of gap junctions inhibits the ion transfer between pigmented epithelial (PE) and NPE cells, regardless of the mechanism of regulation. However, this is unlikely because the biphasic response of ouabain on \(I_{\text{sc}}\) was preserved even in a heptanol-treated preparation. Our result was in agreement with a study in which cAMP was shown to uncouple the gap junctions in rabbit corneal epithelium.40 It has been shown that isoproterenol and forskolin induce a rapid phosphorylation of connexin43 located between PE and NPE cells.41 Whether this is associated with the regulation of gap junction permeability is unclear and awaits further investigation.

In our study, cAMP inhibited the net Cl\(^-\) transport across the ciliary epithelium, potentially reducing the formation of aqueous humor. The effect of cAMP is probably linked to the regulation of permeability of intercellular gap junctions between PE and NPE cells.

**Acknowledgments**

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**References**


