Regulation of gap junction coupling in bovine ciliary epithelium

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The intraocular pressure (IOP) depends directly on the rate of inflow of aqueous humor and the resistance to outflow from the eye. Lowering IOP is the only approach proven to delay the onset and slow progression of glaucomatous blindness (15, 16, 34, 46), so that reducing the inflow rate is a strategy in treating glaucoma.

The ciliary epithelium secretes the aqueous humor. Solute, primarily Na+ and Cl-, and water are transferred from the extracellular stroma of the ciliary processes to the posterior chamber by sequential passage through the pigmented ciliary epithelial (PE) cells, gap junctions, and nonpigmented ciliary epithelial (NPE) cells (Fig. 1). Na+ is ejected through Na+-K+-activated ATPase, and Cl- is released through Cl- channels at the basolateral surface of the NPE into the aqueous humor. Water is released at that surface through aquaporin AQP1 and AQP4 channels (10, 45). The aquaporins at the apical surface of the NPE cells may provide an uptake pathway for water under conditions when the virtual space between the PE and NPE cells accumulates fluid (42). Aquaporins have not yet been identified in PE cells (11).

Of the many potential physiologic regulators of ciliary epithelial secretion, 3’,5’-cyclic adenosine monophosphate (cAMP) has been most intensively studied (21), and blockers of cAMP formation are clinically used to reduce inflow and thereby IOP (3, 47). The antagonists of β-adrenergic receptors used for this purpose do reduce both cAMP production and IOP, but whether the relationship is causal has long been uncertain (5, 36, 38, 52). Unexpectedly, inflow has been reported to be reduced by either increasing (5, 36) or decreasing (3, 47) cAMP production. Perhaps related to this apparent contradiction are reports that cAMP acts on multiple transport components that could favor either stimulation or inhibition of the secretory rate (10, 21). The direct effect of cAMP has long been under study with in vitro ciliary epithelia (9). On the basis of transepithelial measurements of bovine ciliary epithelium, the proposal has been made that cAMP possibly uncouples the intercellular gap junctions between PE and NPE cells (23). Substantial, cAMP-triggered inhibition of gap junctional transmission would be expected to markedly reduce inflow (Fig. 1). However, connexin 43 (Cx43) is well recognized to form a major component of the PE-NPE gap junctions in this tissue (4, 13, 14, 50), and cAMP has generally been found to increase or not affect Cx43 gap junctional communication in other preparations (33, 35, 51).

In the present work, we have directly studied the effect of cAMP on electrical and dye communication in excised native bovine PE-NPE coupled pairs and have also estimated the contribution of Cx43 to PE-NPE communication by small interfering RNA (siRNA) knockdown.

MATERIALS AND METHODS

Cellular model. The previously described procedure for cell isolation and preparation (24) was slightly modified to increase the yield of PE-NPE cell coupled pairs. Briefly, fresh bovine eyes were obtained from a local abattoir. After removal of the cornea and iris along the limbus, ciliary processes were excised in small pieces and rinsed with Dul-
becco’s phosphate-buffered saline (PBS; Invitrogen-GIBCO, Grand Island, NY). The dissociated PE-NPE cell couplets were obtained by incubating the preparation with 0.15% trypsin in a shaker for 20 min at 200 rpm and 37°C. PE cells were readily distinguished from NPE cells by the presence of abundant pigment granules. The cells were washed twice with PBS and plated on coverslips (Fisher Scientific, Pittsburgh, PA) in medium 199 (Invitrogen-GIBCO) for at least 2 h at 37°C in 5% CO2 before the experiment. The medium contained 10% fetal bovine serum and 0.1% gentamicin (Invitrogen-GIBCO). The bovine preparations were prepared 1.5 h after death.

**Patch-clamp measurements.** Micropipettes for whole cell measurements were prepared using a Flaming/Brown micropipette puller (P-97; Sutter Instrument, San Raphael, CA). Micropipettes were fire polished with a microforge (MF-830; Narishige, Tokyo, Japan) to resistances of 3–7 M\( \Omega \). The micropipettes were advanced to the cell surfaces with piezoelectric micromanipulators (models PCS-5400 and PCS-6200; Burleigh, Victor, NY). Couplets chosen for patching displayed a relatively large area of contact between the PE and NPE cells. Cell pairs with minimal areas of contact were avoided in the expectation that such couplets might have arisen from randomly associated cells following harvesting, and were less likely to arise from physiologically coupled pairs in vivo.

To minimize mechanical stress to the cell couplets, we initially tried to conduct whole cell measurements in the perforated-patch mode (31, 41). However, in contrast to experience with certain other cells, both \( \text{escin} \) (26) and gramicidin (1) caused death of the ciliary epithelial cells. As we have previously found (2), perforated patches can be formed with amphotericin or nystatin. However, the probability of success was so low with these cells, that forming perforated patches with both cells of the NPE-PE couplets was deemed impossible. Instead, ruptured patches were formed after first establishing gigohm seals in both cells of the couplet.

Currents in couplets of PE and NPE cells were measured by dual whole cell patch clamping (49) with a Multiclamp 700B (Axon Instruments, Foster City, CA) coupled to an external Bessel filter (model 900; Frequency Devices, Haverhill, MA). Data were acquired and analyzed with a digital interface (Digidata 1220 with Clampex 9 software; Molecular Devices, Union City, CA). At the beginning of each experiment, both cells were clamped at the same holding potential (0 mV) to eliminate any transjunctional electrical driving force. Then one of the cells (the donor cell) was stepped to different voltages (from either \(-110 \text{ mV} \) to \(+110 \text{ mV} \) or from \(-80 \text{ mV} \) to \(+80 \text{ mV} \), in 20-mV increments). The junctional current values were determined by averaging the current in the recipient cell at \(+10 \text{ mV} \) throughout the entire voltage step.

**Lucifer Yellow dye transfer.** The PE cell of each PE-NPE couplet was chosen to be the donor cell and was patched with a micropipette containing Lucifer Yellow (1 mg/ml) (48) for 10 min. Diffusion of the dye into the NPE cell was used to determine the extent of gap junction coupling. The Lucifer Yellow dye was taken up by the NPE cell and visualized by fluorescence microscopy. The extent of dye transfer was quantified by measuring the fluorescence intensity in the NPE cell.

### Table 1. Composition of micropipette filling solutions and external bath

<table>
<thead>
<tr>
<th>Component</th>
<th>Voltage Clamp</th>
<th>Dye Transfer</th>
<th>External Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>25.0</td>
<td>25.0</td>
<td>116.0</td>
</tr>
<tr>
<td>NMDG</td>
<td>120.0</td>
<td>110.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>25.0</td>
<td>25.0</td>
<td>116.0</td>
</tr>
<tr>
<td>Aspartate</td>
<td>110.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
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<td>1.0</td>
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</tr>
<tr>
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<td>0.38</td>
<td>1.8</td>
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<tr>
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<tr>
<td>Glucose</td>
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<td>5.0</td>
</tr>
<tr>
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<td>0.0</td>
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</tr>
<tr>
<td>EGTA</td>
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<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>ATP</td>
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<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.01</td>
<td>0.0</td>
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<tr>
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<td>7.2</td>
<td>7.4</td>
</tr>
<tr>
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<td>278</td>
<td>309</td>
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</table>

NMDG, N-methyl-D-glucamine. \(^*\)Free Mg\(^{2+}\) concentration was 260 \(\mu\)M in micropipette. Except for pH and osmolality, all values are in mM. \(^\dagger\)Free Ca\(^{2+}\) concentration was 100 nM in micropipette.
dye was imaged at 5 or 10 min after break-in, using a digital camera (Nikon coolpix C4500, Japan) connected to a computer. The fluorescence intensities in recipient cells, donor cells, and background were analyzed by ImageJ software (National Institutes of Health, Bethesda, MD). The total measured intensities were corrected by subtracting the background intensities. Relative intensities were calculated as the ratio of the corrected fluorescent intensities of the recipient to the source cell.

Gene expression analysis. Connexin 40 (Cx40), Cx43, and peptidylprolyl isomerase B (PPIB) mRNA expression was analyzed by quantitative polymerase chain reaction with reverse transcription (qRT-PCR) using Taqman Gene Expression Assays (Applied Biosystems, Foster City, CA). Total RNA from bovine ciliary epithelial cells, kidney medulla, and cerebral cortex was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA) and reverse transcribed using Taqman Reverse Transcription reagents (Applied Biosystems) according to the manufacturers’ protocols. The TaqMan primers and probes (Applied Biosystems) used were as follows: Cx40 forward primer, GGTCTGCCAGTCATCC; Cx40 reverse primer, ACGTTCGCAAACGAGTT; Cx40 probe, TATACAGAGGAAATCAGC; Cx43 forward primer, TGCAGACTGAGTGCCTTA; Cx43 reverse primer, GCCGT-GAGATGCGGACTGTTGAA; Cx43 probe, CTGGCTACAGAGTTTGGCC; PPIB forward primer, CCCATGGAACCTCAGTTAAC; PPIB reverse primer, GAGGTTGTTGCTTGGC; PPIB probe, TTGGCATGTCACCC. qRT-PCR was performed using the TaqMan Universal PCR Master Mix and the Prism 7500 sequence detection system (Applied Biosystems). The thermal cycling protocol for the PCR was 50°C for 2 min and 95°C for 10 min, followed by 40 repeat cycles of 95°C for 15 s and 60°C for 1 min. Cx40 and Cx43 mRNA expression was normalized to expression of an endogenous control (PPIB). The relative quantification values (RQ) of target-gene knockdown were calculated with the $2^{-\Delta\DeltaCT}$ method, where CT is threshold cycle.

siRNA knockdown. Freshly isolated primary bovine PE and NPE cells were transfected at 30 nM siRNA using XtremeGene (Roche, Indianapolis, IN) at a 5:1 XtremeGene:siRNA ratio immediately after the trypsinized cells were plated on coverslips. The siRNA against bovine Cx43 (siCx43) was a Custom SmartPool (Dharmacon, Lafayette, CO) comprising individual siRNAs targeting the sequences GAGCAAA-CUGGGCCAAUU, CUGAGAACCUACA-UCAUCA, UAGGCAA-ACUCUUGACAA, and GUACCUGGCUAGUGUUC. Nontargeting control siRNA no. 1 (NTC1) was used as a negative control (Dharmacon).

Fig. 2. Effect of heptanol on junctional currents of a PE-NPE cell couplet. Following the voltage protocol displayed at bottom, voltages applied to the stepped cell (cell 1) produced junctional currents recorded in the recipient cell (cell 2) (A). Currents in both cells were stable when measured under control conditions at points during the subsequent 16 min (B–D). Perfusion with 4.3 mM heptanol for 2.5 min abolished the junctional currents (E), but currents in both the stepped and recipient cells were restored after subsequent washout for 7.5 min (F). t, Time.
Cx43 expression was determined by qRT-PCR and Western blot at 24–48 h posttransfection. Antibodies against Cx43 and tubulin were obtained from BD Biosciences (San Jose, CA) and Abcam (Cambridge, MA), respectively. Western immunoblots were scanned and bands were quantified with the ImageJ software.

**Chemicals and solutions.** All chemicals were reagent grade. H-89 was purchased from Biomol (Plymouth Meeting, PA) and dibutyryl-cAMP from Sigma-Aldrich (St. Louis, MO). The compositions of the solutions in the micropipettes and external bath are provided in Table 1. Bicarbonate was omitted to obviate potential pH-dependent changes in gap junctional permeability triggered by redistributions of CO2 and HCO3−. Potassium was omitted to minimize uncontrolled shifts in plasma-membrane conductance, in part through cAMP-stimulated K+ channels of NPE cells (12). Solutions were filtered through 0.22 μm (Millipore) before use.

**Statistical analysis.** Student’s t-test, paired or unpaired as appropriate, was applied in comparing two sets of data, and one-way ANOVA was applied to compare three sets of data. Statistical analyses were performed with SigmaStat (Aspire Software International). Unless otherwise stated, the results are presented as means ± SE. P < 0.05 was considered statistically significant.

**RESULTS**

**Dual-cell patch clamping.** As illustrated by Fig. 2A, voltages applied to the stepped cell (cell 1) produced junctional currents in the recipient coupled cell (cell 2). Dual-patch clamping of PE-NPE cell couplets revealed a wide range of junctional conductances, with a mean ± SE of 6 ± 3 nS (n = 6, 20 mV). The fractional contribution of the junctional currents to the total currents was likewise variable, presumably reflecting variation in the numbers of gap junctions coupling the PE and NPE cells. For example, the baseline junctional currents con-

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**Figure 3.** Effects of dibutyryl-cAMP, H-89, and heptanol on junctional currents of PE-NPE cell couplets. Currents in the stepped cell (cell 1; A–F, bottom) and in the recipient cell (cell 2; A–F, top) were recorded during the course of perfusing cAMP (500 μM dibutyryl-cAMP) and heptanol (4.3 mM) sequentially with or without H-89 (2 μM) in separate couplets. H-89 prevented the inhibitory effect of cAMP.
tributed ~50% to the total currents measured in the stepped cell of the couplet of Fig. 2A, and ~80% of that measured in the couplet of Fig. 3A. The junctional currents shown in Fig. 2, A–D, were stable when measured under control conditions during a period of 14 min but were abolished after perfusion with 4.3 mM heptanol (Fig. 2E). The currents in the stepped cells were correspondingly reduced, with the residual currents proceeding across the plasma membrane. Heptanol is a commonly used blocker of connexin gap junctions (8, 44). The response to heptanol documented that the currents measured in the recipient cells passed through the gap junctions and not through cytoplasmic strands. The inhibition produced by heptanol was largely reversible (Fig. 2F).

Under our experimental conditions, perfusion with a membrane-permeant form of cAMP (500 μM dibutyryl-cAMP) reduced junctional currents within approximately 5 min. After 10 min, these currents were reduced by 54 ± 8% (n = 6, P < 0.005, t-test), measured at a step potential of +10 mV, and by 42 ± 10% (n = 6, P < 0.01), measured at −10 mV (Figs. 3 and 4). In contrast, blocking cAMP-activated kinase (PKA) with H-89 (7) prevented the cAMP-triggered reduction in currents (n = 6). In the experiments of Figs. 3 and 4, couplets were perfused with 2 μM H-89 for 15 min before applying the cAMP in the continued presence of 2 μM H-89. Perfusion with 4.3 mM heptanol at the conclusion of the dual patch-clamp experiments abolished the junctional currents. Perfusion with H-89 alone did not significantly affect baseline junctional currents at either +10 or −10 mV (one-way ANOVA).

Lucifer Yellow dye transfer. Parallel studies of gap junctional permeability were conducted by monitoring dye transfer. As illustrated by Fig. 5, Lucifer Yellow fluorescence was detected shortly after breaking into the donor cell. Transfer of dye proceeded more slowly over the succeeding 10 min. Figure 6 summarizes the relative intensities of fluorescence in recipient and donor cells measured 10 min after initiating measurement, and corrected for background fluorescence. As in the case of the junctional currents, 500 μM dibutyryl-cAMP reduced the rate of Lucifer Yellow dye transfer. Measured after 10 min, the cAMP lowered the fluorescence ratio from 0.41 ± 0.05 (n = 15) in control couplets to 0.17 ± 0.05 (n = 20), an inhibition of 59 ± 19%. In parallel experiments, perfusion with 4.3 mM heptanol reduced the fluorescence ratio to 0.06 ± 0.02 (n = 6). One-way ANOVA and pairwise comparisons with the Tukey test indicated that the inhibitions produced by both cAMP and heptanol were significant at the 0.002 probability level. The inhibitions produced by cAMP and heptanol were not significantly different from each other.

Expression of Cx43 and Cx40. qRT-PCR indicated that bovine ciliary epithelium expressed Cx43 at least 100-fold more than Cx40 under baseline conditions (Fig. 7). Positive and negative controls for the Cx40 primers were provided by measurements conducted on RNA from kidney and brain, respectively (Fig. 7), conforming to the known organ distribution of Cx40 (e.g., Refs. 17 and 29).

Knockdown of Cx43. Transfection of harvested bovine ciliary epithelial cells with siCx43 inhibited Cx43 mRNA expression by 55 ± 7% after 24 h (n = 4, P < 0.001) relative to cells transfected with the NTC1 control siRNA (Fig. 8). As illustrated by Fig. 9, siCx43 also reduced expression of Cx43 protein in replicate experiments. Normalizing the intensities to those of the untreated controls, and calculating the uncertainty as one-half of the difference between the band intensities, the mean values were 18 ± 8% (siCx43), 105 ± 11% (NTC1 control), and 84 ± 16% (−siRNA control). Correspondingly, siCx43 knockdown reduced functional gap junctional communication, as determined by Lucifer Yellow dye transfer between PE and NPE cells 48 h after transfection (Fig. 10). The ratio of fluorescence intensity in recipient to donor cell was 0.47 ± 0.09 (n = 11) 10 min after whole cell patch formation in PE-NPE couplets transfected with the NTC1 control siRNA. In contrast, siCx43 siRNA decreased the fluorescence intensity ratio to 0.20 ± 0.01 (n = 13), an inhibition of ~60% (P < 0.02) (Fig. 10). The similarity of the qRT-PCR (Fig. 9) and Western immunoblot results (Fig. 10) obtained with nontreated and nontargeted control cells suggests that the siRNA did not affect viability.

**DISCUSSION**

The salient results of the present study are that 1) under certain conditions, dibutyryl cAMP can acutely reduce gap junctional communication between PE and NPE cells, 2) the inhibitory action of cAMP can be entirely prevented by blocking PKA activity, and 3) knockdown of endogenous Cx43 reduces gap junctional permeability.

**Opposing actions of cAMP on aqueous humor inflow.** The data support the suggestion, based on transmural measure-
ments, that cAMP partially uncouples gap junctions linking PE and NPE cells (23). In contrast to its direct activation of maxi-Cl\(^-\) channels of PE cells (24, 27), cAMP can lower gap junctional communication indirectly by activating PKA, at least under our experimental conditions. This observation adds to the literature documenting that cAMP exerts actions expected both to stimulate and inhibit net aqueous humor formation (10, 21). Stimulation by cAMP of the Na\(^+\)-K\(^+\)-2Cl\(^-\) symport at the stromal surface (18) and of Cl\(^-\) channels (6, 25) and Na\(^+\)-K\(^+\)-activated ATPase (37) at the aqueous surface should enhance secretion. In contrast, cAMP-triggered activation of the maxi-Cl\(^-\) channels at the stromal surface (23) and acute inhibition of PE-NPE gap junctions should reduce net secretion. Under certain conditions, cAMP has also been reported to inhibit Na\(^+\)-K\(^+\)-activated ATPase of NPE cells (19, 40), which would likewise reduce the rate of aqueous humor formation. The predominant integrated effect of cAMP may be determined by compartmentation (21). Such compartmentation has been documented by Huang et al. (32), who found that 1 µM adenosine increased local cAMP concentration sufficiently to activate CFTR Cl\(^-\) channels of Calu-3 airway cells while elevating the total cAMP content very little.

**Connexin composition of PE-NPE gap junctions.** Cx43 has been the only connexin consistently and rigorously identified in ciliary epithelium of multiple species and localized to PE-NPE junctions (4, 13, 14, 50). Despite early evidence implicating its presence in rabbit and rat preparations (50), later work indicated that Cx50 is not expressed in rat PE-NPE gap junctions (14). However, recent preliminary immunoblot and immunolocalization evidence has suggested that Cx50 is expressed on the basolateral surface of bovine NPE cells, contralateral to the PE-NPE gap junctions (43). Coffey et al. (14) also found evidence for Cx40 in the PE-NPE gap junctions of rat ciliary epithelium, using RT-PCR and antiserum developed by Dr. R. Gourdie (Medical University of South Carolina, Charleston, SC). In contrast, Calera et al. (4) were unable to detect Cx40 expression in mouse ciliary epithelium, using antibody purchased from Alpha Diagnostic International (San Antonio, TX) under conditions thought to minimize nonspecific staining. The present qRT-PCR measurements indicate that Cx40 expression

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**Fig. 5.** Transfer of Lucifer Yellow dye through gap junctions of a PE-NPE cell couplet. Transfer of the fluorescent dye from the donor to the recipient cell was observed as early as 1 min after break-in. A bright-field photomicrograph of the PE-NPE couplet taken at the conclusion of the experiment is included at bottom.
of native bovine ciliary epithelial cells is no more than 1% of the Cx43 expression. These data are consistent with our functional observations that partial knockdown of message for Cx43 both reduced expression of Cx43 protein and produced large, comparable inhibitions of junctional currents (∼50%) and of Lucifer Yellow dye transfer (∼60%).

We conclude that Cx43 is a consistent major component of PE-NPE gap junctions and that Cx40 may have a more limited species distribution. This view is consistent with increasingly evident species variation in expression of other ciliary epithelial transport activities (22, 28).

Gap junctional target site of PKA. The target site of phosphorylation by PKA is unclear. Cx43 is known to be phosphorylated by protein kinase C, MAP kinase, and the pp60src kinase (35). Although Cx43 might also be directly phosphorylated by PKA, most evidence argues against this view (20, 35). Furthermore, in contrast to the current observations, cAMP commonly increases Cx43 gap junctional permeability in other preparations (20, 35, 51), albeit reducing message for Cx43 in rat Leydig cells (53). In addition, intracellular cAMP had no acute effect on Cx43 and Cx40 gap junctions expressed in HeLa cells (33). One possible interpretation of the published data and our own results is that PKA might mediate rapid actions of cAMP, by targeting a regulatory protein not ubiquitously expressed in all gap junctions.

Potential gap junctional regulation by cAMP. The present results suggest that cAMP may trigger inhibition of gap junctions in some species. It is unclear whether cAMP is delivered

![Fig. 6. Effects of dibutyryl-cAMP and heptanol on Lucifer Yellow (LY) transfer through PE-NPE gap junctions. In comparison to control couplets (n = 15), cAMP (n = 20) and heptanol (n = 6) reduced gap junctional permeability (*P = 0.002, ANOVA) in parallel experiments. In this figure and Fig. 10, the dye transfer has been quantified as the mean intensity ratio (± SE) in the recipient to the donor cell.](image)

![Fig. 7. Relative quantification of message for connexin 43 (Cx43) and Cx40 by quantitative RT-PCR (qRT-PCR). Values are presented as means ± SD. RNA was prepared from duplicate samples extracted from bovine kidney and brain and from a single harvest of bovine ciliary epithelial cells. Each value was averaged from four real-time qRT-PCR measurements. Message detected in ciliary epithelial cells for Cx43 was two orders of magnitude larger than that for Cx40. Message for the widely distributed Cx43 was also detected in bovine kidney and brain. However, as expected, Cx40 was substantially expressed in kidney, but not in brain.](image)

![Fig. 8. Effect of small interfering RNA (siRNA) knockdown on relative quantification of message for Cx43 by qRT-PCR. Values are presented as means ± SD. Control cells were nontreated (control), treated with nontargeting control siRNA (NTC1), or treated with lipofectant alone (−siRNA). Relative to NTC1, transfection with siRNA directed against Cx43 (siCx43) reduced the message after 24 h (n = 4, *P < 0.001).](image)
to the putative modulatory sites in sufficiently high concentrations to regulate PE-NPE gap junctional permeability and the rate of aqueous humor inflow in vivo. Intracellular cAMP is functionally compartmentalized (32), with cyclic nucleotide phosphodiesterases tending to limit the signal transduction through cAMP diffusion. We have been relating our findings to published studies of β-adrenergic agonists and antagonists and of forskolin, all of which alter cAMP production by membrane-bound adenyl cyclase. However, the soluble bicarbonate- and Ca2+-dependent adenyl cyclase (sAC) is present in bovine ciliary processes (39) and may also generate cAMP for delivery to the PE-NPE gap junctions. Recent work suggests that sAC is a physiological regulator of Na+ transport by renal cortical collecting duct cells (30). Whether sAC plays a comparable role in cAMP-dependent regulation of gap junctional permeability and of aqueous humor inflow remains to be determined.

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DISCLOSURES

One or more authors is employed by and/or has a financial interest worth more than US $10,000 (from consultancy honoraria/expert testimony/corporate grants/patents pending/royalties/other) and/or has a 5% equity in an entity related to the subject matter discussed in the article. Our article reports collaborative results, in part utilizing an siRNA knockdown strategy in bovine ciliary epithelial cells. That strategy is incorporated in a pending patent submitted by Alcon to lower intraocular pressure. Three of the coinventors listed on the patent application are J. E. Chatterton, A. F. Clark, and M. B. Wax while all three were employees of Alcon. J. E. Chatterton remains an employee of Alcon. A. F. Clark and M. B. Wax have since left Alcon. All three former or current employees (J. E. Chatterton, A. F. Clark, and M. B. Wax) retain stock options in Alcon. The decision to publish the results was entirely that of the authors, and not that of Alcon.

REFERENCES

GAP JUNCTION COUPLING IN BOVINE CILIARY EPITHELIUM


