Release of ATP from retinal pigment epithelial cells involves both CFTR and vesicular transport

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Submitted 26 April 2004; accepted in final form 26 August 2004

Reigada, David, and Claire H. Mitchell. Release of ATP from retinal pigment epithelial cells involves both CFTR and vesicular transport. Am J Physiol Cell Physiol 288: C132–C140, 2005. First published September 15, 2004; doi:10.1152/ajpcell.00201.2004.—The retinal pigment epithelium (RPE) faces the photoreceptor outer segments and regulates the composition of the interstitial subretinal space. ATP enhances fluid movement from the subretinal space across the RPE. RPE cells can themselves release ATP, but the mechanisms and polarity of this release are unknown. The RPE expresses the cystic fibrosis transmembrane conductance regulator (CFTR), and CFTR is associated with ATP release in other epithelial cells. However, an increasing number of reports have suggested that the exocytotic pathway contributes to release. In the present study, we examined the involvement of CFTR and the vesicular pathway in ATP release from RPE cells. Release from cultured human ARPE-19 cells and across the apical membrane of fresh bovine RPE cells in an eyecup was studied. A cAMP cocktail to activate CFTR triggered ATP release from fresh and cultured RPE cells. Release from both RPE preparations was largely prevented by the broad-acting blocker glibenclamide and the specific thiazolidinone CFTR inhibitor CFTR-172. The block by CFTR-172 was enhanced by preincubation and prevented ATP release with 3.5 μM IC50. The rise in intracellular Ca2+ accompanying hypotonic challenge was prevented by CFTR-172. The vesicular transport inhibitor brefeldin A prevented ATP release after stimulation with both hypotonic and cAMP conditions, suggesting vesicular insertion was also involved. These results show an intimate involvement of CFTR in ATP release from RPE cells which can autostimulate receptors on the apical membrane to modify Ca2+ signaling. The requirement for both CFTR and vesicular transport pathways suggests vesicular insertion of CFTR may underlie the release of ATP.

Cystic fibrosis transmembrane conductance regulator; recycling endosomes; brefeldin A; autostimulation; retinal detachment

The retinal pigment epithelium (RPE) is a monolayer of cells lying between the retinal photoreceptors and the choroidal blood supply. The RPE functions in a glial cell-like capacity to maintain the health and signaling ability of the outer retina. The RPE supplies the outer retina with general nutrients and components critical for the visual cycle (10). The distal tips of the photoreceptor outer segments are regularly phagocytosed and metabolized by the RPE to maximize photoreceptor responsiveness (66). The RPE also regulates the ionic composition of the extracellular subretinal space surrounding the outer segments and controls the absorption of fluid from the retina to the choroid (13, 22, 32, 34, 35).

This complex interaction between the RPE and the photoreceptors is dependent on close communication between the two cell types. Various neurochemicals have been implicated in this communication, including dopamine (15, 59), serotonin (39), and epinephrine (13, 24, 42). The purines ATP and adenosine also contribute to RPE-retina interaction. Stimulation of the RPE by adenosine can modify the rate of outer segment phagocytosis (17), while stimulation of apical P2Y2 receptors by ATP can trigger elevation of Ca2+ (40, 55).

However, the mechanisms involved in this release remain to be determined. Previous work has shown that while the Ca2+ ionophore ionomycin is not sufficient to trigger release, the general Cl− channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoate inhibits it, suggesting anion channel involvement (36). RPE cells have been shown to express the anion channels CIC-2, CIC-3, CIC-5, the pCLCA1 Cl− channel regulator, and the cystic fibrosis transmembrane conductance regulator (CFTR; Refs. 4, 26, 63, 64). Of these, ATP release has been associated most consistently with CFTR (7, 41, 52, 60). However, recent evidence suggests that the exocytotic pathway may also be important, because substances that interfere with vesicular transport, such as botulinum toxin, tetanus neurotoxin, and brefeldin A (BFA) can prevent stimulated ATP release (5, 30, 58). Consequently, we investigated the involvement of CFTR in ATP release from RPE cells and examined whether vesicular transport contributed to this release. Preliminary results were presented previously in abstract form (43, 44).

MATERIAL AND METHODS

Cell culture. ARPE-19 cells (12) were obtained from the American Type Culture Collection (Manassas, VA) and grown in 25-cm2 Falcon primary culture flasks (Becton Dickinson, Franklin Lakes, NJ) in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium with 3 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 mg/ml Fungizone, and/or 50 μg/ml gentamicin (all obtained from Invitrogen, Carlsbad, CA) in the presence of 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT). Cells were incubated at 37°C in 5% CO2 and subcultured weekly with 0.05% trypsin and 0.02% EDTA.

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Solutions. The isotonic solution used as a basis for all solutions was composed of (in mM) 105 NaCl, 5 KCl, 6 HEPES acid, 4 Na-HEPES, 5 NaHCO₃, 60 mannitol, 5 glucose, 0.5 MgCl₂, and 1.3 CaCl₂, pH 7.4. Hypotonic solution was obtained by adding the required amount of deionized distilled water, with "hyperosmotic" defined as the percentage of water added. Giblencilamide, forskolin, 3-isobutyl-1-methylxanthine (IBMX), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N"-tetraacetic acid (BAPTA), and CFTR-172 were mixed as stock solutions in dimethyl sulfoxide. The CFTR-172 was a kind gift from A. S. Verkman (Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA). The cAMP-stimulating mixture contained (in µM) 10 forskolin, 100 IBMX and 500 8-4-chlorophenylthio(cpt)-cAMP. Brefeldin A was dissolved in ethanol. All chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

ATP measurements from cultured cells. ATP release was detected through the chemiluminescent luciferin-luciferase reaction, and the light emitted was recorded using a microplate luminometer (Lumicon, Franklin, MA). ARPE-19 cells were grown to confluence in 96-well white assay plates with clear bottoms. For ATP measurements, the cells were washed, cells were loaded with 10 µM fura-2 and 0.2% pluronic acid (Molecular Probes, Eugene, OR). The field of 20–30 cells was alternatively excited at 340 and 380 nm with a monochromator (Photon Technologies International, Lawrenceville, NJ), and the fluorescence emitted at 510 nm was detected with a photometer (Photon Technologies International). The ratio of light detected after excitation at 380 and 340 nm was converted into Ca²⁺ concentration using a calibration performed after each measurement as previously described (36).

Data analysis. Baseline levels of ATP varied considerably by experimental day for both fresh and cultured experiments. Efforts to remove this variability, such as limiting cell age and changing the solution 1 h before the measurements, were only partially effective, and the raw concentrations throughout the text reflect this variation. To allow comparison of experiments, values were normalized to the mean control value of each day. All data are expressed as means ± SE, and the unpaired Student’s t-test was used for statistical analysis, with P < 0.05 defined as significantly different. The IC₅₀ of CFTR-172 and the EC₅₀ of hypotonicity were determined by fitting the data curve with first-order exponential curves using SigmaPlot graphing software (SPSS, Chicago, IL).

RESULTS

Fresh vs. cultured cells. While the cultured human ARPE-19 cells have many advantages, it was important to determine the characteristics of ATP release by fresh RPE cells and the polarity of any such release. In the RPE eyecup preparation, the apical membrane faces the cup interior, and both drug access and ATP release likely occur across the apical membrane. Initial experiments thus compared the ATP release characteristics from cultured ARPE-19 cells and the fresh bovine RPE eyecup stimulated with hypotonic solution.

ATP release from ARPE-19 cells reached a peak 10 min after the addition of a 50% hypotonic solution, after which levels slowly declined for the remainder of the 30-min measurement period (Fig. 2A). Peak levels rose more than eightfold in this series of experiments, from 1.7 ± 0.5 nM to 14.1 ± 1.6 nM (n = 8 for both). The amount of ATP present in the well rose with the degree of hypotonicity >30% and reached a peak at 50% hypotonicity. Increasing hypotonicity to 60% did not...
produce any additional increase, giving an EC50 of ~42% (Fig. 2B).

The release from fresh bovine cells after hypotonic challenge was similar to that of the ARPE-19 cells. Because the peak from ARPE-19 cells occurred at 10 min, samples were taken from the intact bovine RPE eyecup at 10 min. Levels showed that the release across the apical membrane of fresh cells was increased significantly when challenged with 30% hypotonicity. Presentation with a 50% hypotonic solution raised ATP levels from 7.8 ± 0.6 nM (n = 6) to 22.2 ± 2.8 nM (n = 5). The ATP release pattern from fresh and cultured cells is shown in Fig. 2C. Later experiments used the minimum level of hypotonicity that reproducibly induced a significant release in each system: 30% for the fresh bovine RPE cells and 50% for the ARPE-19 cultured cells.

Release triggered by cAMP. To determine whether CFTR was involved in ATP release from RPE cells, the effect of intracellular cAMP was examined because activation by the cAMP-dependent protein kinase A is typically necessary for the opening of the CFTR conductance pathway (2). Intracellular cAMP was increased with a cAMP cocktail containing cell-permeant cpt-cAMP, forskolin, and IBMX. The cocktail triggered an immediate increase in ATP levels bathing ARPE-19 cells (Fig. 3A). While recording from the assay plate generally began within 60–90 s after addition of stimuli, continuous monitoring during cocktail addition confirmed that ATP levels reached their peak within 30 s. Peak levels of ATP rose fourfold when cells were exposed to the cocktail (Fig. 3B). Although both forskolin and cpt-cAMP increased bath levels of ATP, levels were highest with the complete cocktail. Addition of the cocktail at the peak of the hypotonic response did not
lead to a further increase. In fresh bovine RPE cells, the cAMP-stimulating cocktail increased ATP levels 2.5-fold, from $10.1 \pm 2.1$ nM ATP ($n = 6$) to $24.3 \pm 9.3$ nM ATP ($n = 6$, Fig. 3C). Forskolin itself led to a small but not significant increase (Fig. 1), it was not possible to reliably quantify its enhancement.

CFTR blockers. Pharmacological tools were used to further elucidate the contribution of CFTR to ATP release from RPE cells. Because glibenclamide can block the Cl$^-$ current associated with CFTR (65), its effects on ATP release were determined. When glibenclamide (100 nM) and hypotonic solutions were added together to the ARPE-19 cells, a reduction in ATP was seen throughout the experiment (Fig. 4A). In this series of experiments, hypotonicity increased peak ATP levels 8.9-fold, from $3.3 \pm 1.1$ nM ($n = 6$) to $29.1 \pm 3.8$ nM ($n = 7$). Glibenclamide blocked 71% of the peak response, with levels of $9.7 \pm 1.5$ nM ($n = 6$). A summary of the effects of 100 nM glibenclamide from all trials is shown in Fig. 4B. The drug produced a complete block of ATP release from bovine RPE cells when used at 100 nM (Fig. 4C). Hypotonicity raised ATP 2.2-fold in the bovine RPE eyecup, from $0.26 \pm 0.02$ nM ($n = 6$) to $0.35 \pm 0.04$ nM ($n = 7$). In the presence of 100 nM glibenclamide, hypotonicity raised ATP to only $0.13 \pm 0.02$ nM ($n = 5$; not significantly different from control). Levels were particularly low in this set of experiments, but they were consistent, as evidenced by the small variation.

Although the block by glibenclamide suggested that CFTR contributes to ATP release from the RPE, the drug is not specific for CFTR. A thiazolidinone blocker, CFTR-172, that inhibits CFTR with far greater potency and specificity than glibenclamide was recently identified by Verkman and colleagues (28, 56). CFTR-172 was used to provide a more decisive assessment of the CFTR contribution to ATP release. The blocker CFTR-172 inhibited hypotonically triggered release from ARPE-19 cells (Fig. 5A). When the drug was added simultaneously with the hypotonic solution, minimal block was observed, but inhibition was considerably increased by preincubating the cells with 10 nM CFTR-172 for 30 min (Fig. 5B). Preincubation with 10 nM CFTR-172 decreased the ATP levels bathing ARPE-19 during hypotonic exposure by 79.3%, from $13.4 \pm 1.0$ nM ATP ($n = 23$) to $4.3 \pm 1.2$ nM ATP ($n = 12$), compared with control of $19 \pm 0.1$ nM ($n = 46$). The decrease in ATP levels was concentration dependent, with an IC$_{50}$ of 3.5 nM when preincubated for 30 min. CFTR-172 completely blocked the hypotonically triggered release from fresh bovine cells (Fig 5C). Hypotonicity (30%) raised the peak ATP concentration 1.7-fold, from $8.5 \pm 2.0$ nM ($n = 12$) to $14.9 \pm 3.2$ nM ($n = 8$). CFTR-172 reduced the level by 95%, to $8.9 \pm 3.4$ nM ($n = 8$).

Effect on intracellular Ca$^{2+}$. The levels of extracellular ATP measured after hypotonic or cAMP stimulation in RPE cells are below the EC$_{50}$ for P2Y receptors present on the apical membrane of RPE cells. While the localization of release sites and receptors within microdomains suggests that the effective local concentration of ATP may be considerably greater (21, 25), we sought physiological evidence that the CFTR-linked release of ATP was sufficient to stimulate receptors by examining its effect on intracellular Ca$^{2+}$ levels, as P2 receptor stimulation is known to elevate Ca$^{2+}$ in RPE cells (40).

ARPE-19 cells perfused with hypotonic solution exhibited a two-phase increase in intracellular Ca$^{2+}$, with a large, transient increase followed by a slow rise in Ca$^{2+}$ (Fig. 6A). The inhibitor CFTR-172 greatly diminished both Ca$^{2+}$ responses. Cells exposed to 10 nM CFTR-172 for 30 min before and during hypotonic challenge showed a 95% reduction in the transient peak and a 42% reduction in the delayed Ca$^{2+}$ peak (Fig. 6B). In both cases, a net reduction in Ca$^{2+}$ concentration was observed in response to hypotonicity, possibly because of the dilution of cellular constituents. Because CFTR-172 did not affect levels in isotonic solution or calibration readings, the inhibition was specific for stimulated release of ATP. This implies that CFTR-associated release of ATP is capable of autostimulating receptors on the RPE and suggests that this...
release is responsible for both the fast and slow elevations in Ca\textsuperscript{2+} after hypotonic challenge.

**Involvement of vesicular transport.** While the above experiments with CFTR-172 clearly establish a predominant role for CFTR in the release of ATP, the effects of BFA on ATP release were examined to determine whether the vesicular release pathway was also involved in the response. ARPE-19 cells were preincubated with 10 \mu{M} BFA for 2 h before exposure to a 50% hypotonic solution. The BFA slowed the release of ATP, with a 10-min preincubation reducing release by 79\%, to 2.9 \pm 0.2 \text{nM (n = 60; Fig. 7C).}

Several controls were performed to further explain the dramatic block of ATP release by BFA. While BFA had no effect on the luciferase assay (Fig. 1), the inhibition was clearly dependent on exposure time, with a 10-min preincubation producing a smaller reduction in ATP release. Hypotonicity

![Fig. 5. CFTR-172 blocks release of ATP. A: time-dependent traces showing ARPE-19 cells exposed to 50% hypotonic solution (shaded squares; n = 23), control (open circles; n = 46), and preincubated with 10 \mu{M} CFTR-172 for 30 min before application of hypotonicity (closed inverted triangles; n = 12). B: hypotonically triggered release of ATP is blocked by CFTR-172 in a concentration-dependent way. The peak response is shown from cells exposed to isotonic solution (open squares; n = 31), to hypotonic solution and 10 \mu{M} CFTR-172 applied simultaneously (open inverted triangles; n = 7), or to hypotonic solution plus various concentrations of CFTR-172 preincubated for 30 min (closed circles; n = 23, 6, 14, 14, 14, and 6 experiments with 0, 0.1, 0.3, 3, 10, and 30 \mu{M} CFTR-172, respectively). The preincubated cells were fit with a single exponential decay with EC_{50}=3.5 \mu{M}. C: in the fresh bovine RPE eyecup, CFTR-172 inhibited all of the ATP release triggered by exposure to a 30% hypotonic solution. The cells were preincubated with CFTR-172 for 30 min before exposure to hypotonicity. Bars, means \pm SE; \textit{P} = 0.006, significantly different from control. **P = 0.044, significantly different from hypotonic solution alone.]

![Fig. 6. CFTR-172 blocks autocrine elevation in Ca\textsuperscript{2+}. A: changes in the intracellular Ca\textsuperscript{2+} of ARPE-19 cells perfused with hypotonic solution were inhibited by CFTR-172. A large and fast increase was detected soon after the stimuli presentation (solid line). The inhibitor CFTR-172 (shaded line) completely inhibited the fast peak and significantly blocked the slow rise after a 30-min preincubation at 10 \mu{M} concentration. Inset shows the secondary response on an expanded scale. The lines show a representative experiment using 20 confluent cells each. B: summary of the effects of the inhibitor CFTR-172 on the changes in intracellular Ca\textsuperscript{2+} observed in the ARPE-19 cells by the action of the 50% hypotonic solution. Preincubation with 10 \mu{M} CFTR-172 produced complete inhibition of the fast peak and a significant inhibition of the slow secondary rise. Change in Ca\textsuperscript{2+} is defined as from control baseline to maximum peak for the first event and from the lowest posthypotonicity point to the second maximum for the slow rise. Bars, means \pm SE; n = no. of recordings from 20 cells each. *P < 0.001 vs. hypotonic solution alone; **P = 0.031 vs. hypotonic solution alone.]

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increased ATP levels by 4.9 ± 0.11-fold after a 10-min incubation in BFA (n = 10; P = 0.15, not significantly different from hypotonic alone) (Fig. 7B). Likewise, the reduction in cAMP-triggered release found after a 10-min incubation with BFA was not significant (Fig. 7C). BFA had a similar effect on the baseline release of ATP; preincubation for 10 min reduced basal levels by 13% (from 1.5 ± 0.08 nM; n = 24 to 1.3 ± 0.1 nM; n = 12), while a 2-h preincubation led to a significant reduction of 27% (to 1.1 ± 0.07 nM; n = 12) (Fig. 7D).

BFA also blocked the cAMP-stimulated ATP release across the apical membrane of fresh bovine RPE cells. In these experiments, the cAMP cocktail increased the ATP levels 1.6-fold, to 64.7 ± 12.9 nM (n = 12) from control levels of 46.0 ± 8.8 nM (n = 12). A 30-min incubation with BFA produced a small but not significant reduction in ATP concentration to 52.4 ± 9.7 nM (n = 11) (Fig. 7E).

Because vesicular release is known to require Ca²⁺ (8), the effect of the Ca²⁺ chelator BAPTA on ATP release was examined. Incubation of ARPE-19 cells in cell-permeant BAPTA-AM for 30 min reduced the ATP release triggered by hypotonicity by >77% (Fig. 7F). Levels were increased from 0.55 ± 0.02 nM in control (n = 16) to 5.63 ± 0.82 nM in hypotonic conditions (n = 14; 10.2-fold increase) but fell to only 1.68 ± 0.29 nM in the presence of BAPTA and hypotonicity (n = 16; 1.7-fold increase from control). This indicates that ATP release requires intracellular Ca²⁺ and further supports a role for the vesicular process in the release of ATP from RPE cells.

**DISCUSSION**

In the present study, the potential involvement of CFTR and vesicular transport in the release of ATP from RPE cells was examined using fresh and cultured RPE cells. On the basis of the following observations, we conclude that CFTR can affect RPE physiology through its involvement in ATP release. Stimulating CFTR with a cAMP-activating cocktail led to ATP release from ARPE-19 cells and across the apical membrane of the fresh RPE eyecup. In these preparations, the hypotonicity that triggers ATP release was prevented by both glibenclamide and the specific inhibitor CFTR-172. The elevation of intracellular
Ca\(^{2+}\) that followed a hypotonic challenge was prevented by the blocker CFTR-172. The transport inhibitor BFA and the Ca\(^{2+}\) chelator BAPTA prevented ATP release, consistent with a role for vesicular transport in the process.

CFTR-172 was recently identified as a high-affinity inhibitor of CFTR that does not block volume-sensitive Cl\(^{-}\) channels, Ca\(^{2+}\)-sensitive Cl\(^{-}\) channels, K\(_{\text{ATP}}\) channels, AQP1, AE1, NHE3, or MDR-1 (28). It produces a voltage-independent block and does not affect cAMP production, phosphatase activity, or single-channel conductance, but leads to a reduction in the mean closed time of the channel and may affect channel gating by binding to NBD-1 (28, 56). Inhibition of anion transport by CFTR was maximal 10 min after application of CFTR-172, consistent with the enhanced inhibition of ATP release from RPE cells after preincubation. While the IC\(_{50}\) for ATP release was slightly higher than that found for anion influx or measurements of short-circuit current (28, 56), this is consistent with a more negative membrane potential in RPE cells. In addition, 10 \(\mu\)M CFTR-172 produced maximal block both of ATP release in the present study and of anion flux from thyroid epithelial cells in previous work (28). The effect of CFTR-172 on ATP release provides strong support for a role of CFTR in RPE physiology and is consistent with the idea that the Cl\(^{-}\) and ATP efflux pathways share a common gating mechanism.

The block of ATP release with CFTR-172 also strengthens the link between ATP release and CFTR. CFTR has been associated with ATP efflux since Reisin et al. (45) indicated overexpression of CFTR led to increased ATP efflux. While additional conduits for ATP efflux are likely (11, 18, 47) and release from some cells can clearly occur in the absence of CFTR (18, 19, 37, 61), the activity of CFTR is correlated with ATP release in many cell types (33, 49), and the presence of immunopurified CFTR in lipid bilayers is linked to ATP conductance (9). ATP was initially thought to permeate the CFTR pore used by Cl\(^{-}\), but the precise pathway of ATP movement is now a matter of some debate. Selective elimination of Cl\(^{-}\) and ATP conductance with different site mutations suggests that both CFTR and a separate cofactor are necessary for ATP efflux (23). Anion channel blockers diphenylamine carboxylate and 4,4’-diisothiocyanostilbene-2,2’-disulfonic acid were found to have differential effects on the Cl\(^{-}\) and ATP conductance at the single-channel level, although the two pathways shared a gating mechanism (54). The ability of CFTR-172 to prevent the release of ATP provides further evidence that one gating mechanism controls the passage of both anions (56) and identifies CFTR-172 as an important tool to explore the role of CFTR in release. However, the effects of CFTR-172 on Cl\(^{-}\) and ATP currents must be examined at the single-channel level to provide further insight into the relationship between CFTR and ATP release.

Brefeldin A and CFTR-172 both blocked >70% of the hypotonically triggered release of ATP. This overlap implies that both processes apply to the same population of released ATP and act in series. Freshly synthesized CFTR has a half-life of 16 h (51), but CFTR on the cell surface can be internalized at 5%/min (27). Because a 2-h preincubation with BFA completely inhibited the ATP release from our preparation, this process is unlikely to be dependent on synthesis of new protein but may instead involve recycled material. BFA has traditionally been associated with vesicular transport in the Golgi, but it can also interfere with a range of trafficking events, including transport of recycling endosomes to the plasma membrane. BFA prevented the formation of clathrin-coated buds at endosomes with <30-min incubation and reduced the clathrin-dependent endosomal recycling of transferrin within 10 min (53, 57). The ability of cAMP to stimulate the insertion of CFTR-containing vesicles into the membrane has been hotly debated, with the process occurring in some cells but not in others (3, 38, 62). At least part of the inhibition of ATP release by BFA in RPE cells indicated above is likely to be on the number of CFTR proteins already present in the membrane, because the block was time dependent and was observed in association with baseline levels of ATP released from unstimulated cells. Whether the larger increase in extracellular ATP levels after hypotonic stimulation reflects the insertion of CFTR-containing vesicles into the membrane as seen with other channel types (16, 58) awaits biochemical assessment of CFTR localization in stimulated and unstimulated cells. Regardless of the reason for this phenomenon, the ability of BFA to prevent ATP release by reducing the amount of CFTR reaching the membrane may reconcile opposing “channel” and “vesicular” theories about the release of ATP. Although the vesicular transport pathway has previously been implicated in the release of ATP from non-neuronal cells, the vesicles were assumed to contain transmitters (1, 5, 30, 58). The hybrid mechanism illustrated in Fig. 8 may underlie ATP release from cells in which roles for both anion channels and vesicular transport have been identified.

The release of ATP into the interior of the bovine RPE eyecup is consistent with a functional localization of CFTR to the RPE apical membrane. In the absence of the retina, the eyecup interior represents subretinal space, and ATP sampled...
from the eyecup interior is most likely to have moved from the RPE interior across the apical membrane into the space. CFTR has been immunolocalized to the apical and basolateral membranes of human RPE (4, 63). While release from both membranes has been shown in airway epithelial cells (20), the present study confirms only release across the apical membrane of fresh bovine RPE cells.

It is tempting to assume that the increase in subretinal ATP after the stimulation of apical CFTR would autostimulate P2Y2 receptors and elevate Ca2+ in vivo as shown in Fig. 6. This rise in Ca2+ may open basolateral Ca2+-sensitive Cl− channels and increase retinal to basolateral fluid absorption if the released ATP acts like the P2Y2 agonists (29, 31). The released ATP may also stimulate P2X receptors present on the RPE (46), but because their activation opens a cation channel, stimulation would further increase intracellular Ca2+. The effects of cAMP on the RPE are complex, possibly reflecting species differences or the action of CFTR on the apical and basolateral membranes in addition to other mechanisms (4, 22). The activation of basolateral Cl− conductance after apical autostimulation with ATP would add an additional level of complexity that may underlie some of the variation in effects attributed directly to cAMP. However, the ability of CFTR-172 to alter Ca2+ physiology shows a definitive role for the transporter, and in patients with cystic fibrosis, the fast oscillation of the electrooculogram was significantly reduced (33), suggesting that CFTR function in wild types is regulated by light. It will be interesting to see whether subretinal ATP levels are also affected by light.

ACKNOWLEDGMENTS

We thank W. W. Reenstra for his advice on CFTR pharmacology.

GRANTS

This work was funded by National Eye Institute Grant R01 EY-13424 (to C. H. Mitchell) and National Eye Institute Core Vision Grant EY-01583.

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