The activity of volume-sensitive Cl\(^-\) channels was studied in human tracheal epithelial cells (9HTEo\(^-\)) by taurine efflux experiments. The efflux elicited by a hypotonic shock was partially inhibited by adenosine receptor antagonists, by \(\alpha,\beta\)-methyleneadenosine 5\'-diphosphate (\(\alpha\beta\text{MeADP}\)), an inhibitor of the 5\'-ectonucleotidase, and by adenosine deaminase. On the other hand, dipyridamole, a nucleoside transporter inhibitor, increased the swelling-induced taurine efflux. Extracellular ATP and adenosine increased taurine efflux by potentiating the effect of hypotonic shock. \(\alpha\beta\text{MeADP}\) strongly inhibited the effect of extracellular ATP but not that of adenosine. These results suggest that anion channel activation involves the release of intracellular ATP, which is then degraded to adenosine by specific ectoenzymes. Adenosine then binds to purinergic receptors, causing the activation of the channels. To directly demonstrate ATP efflux, cells were loaded with \([\text{H}]\text{AMP}\), and the release of radiolabeled molecules was analyzed by high performance liquid chromatography. During hypotonic shock, cell supernatants showed the presence of ATP, ADP, and adenosine. \(\alpha\beta\text{MeADP}\) inhibited adenosine formation and caused the appearance of AMP. Under hypotonic conditions, elevation of intracellular Ca\(^{2+}\) by ionomycin caused an increase of ATP and adenosine in the extracellular solution. Our results demonstrate that volume-sensitive anion channels are regulated with an autocrine mechanism involving swelling-induced ATP release and then hydrolysis to adenosine.

Virtually all cells in a multicellular organism undergo swelling or shrinking following changes of intracellular or extracellular osmotic pressure. Although the osmolarity of body fluids, particularly in mammals, is tightly controlled, significant variations may occur in physiological or pathological conditions. For example, a dramatic drop in extracellular osmolarity occurs in the renal medulla during the passage from antidiuresis to diuresis (1). Cell swelling may also occur in the brain during hypoxic and toxic conditions or following treatment of diabetic ketoacidosis (2–5) and in the heart following myocardial ischemia (6, 7). Usually cells respond to osmotic stresses by transporting organic and inorganic osmolytes through the plasma membrane. The lowering of extracellular osmolarity and the consequent cell swelling activate potassium and chloride channels (8). The resulting exit of KCl drives water efflux and therefore restores the original cell volume, a mechanism termed regulatory volume decrease. A key element in this process is represented by volume-sensitive Cl\(^-\) channels, called volume-sensitive organic anion channel (VSOAC), which are also permeable to organic osmolytes such as taurine (9–11). Because taurine in many cells is accumulated at high concentrations through Na\(^+\)-dependent transporters, the opening of VSOAC causes a significant taurine efflux, thus contributing to regulatory volume decrease. The mechanism underlying VSOAC activation is unknown. In a previous work performed on a tracheal epithelial cell line, we found that extracellular ATP is a potent modulator of swelling-induced VSOAC activation (11). This finding led us to test the hypothesis that release of endogenous ATP by the cells could be responsible for VSOAC activation during the hypotonic shock. Our experiments did not support this hypothesis because ATP was not revealed by the luciferin-luciferase method, and exogenous hexokinase did not inhibit VSOAC activity (11). In contrast with our results, other investigators have found that there is a release of ATP induced by cell swelling (12, 13). To reconcile these conflicting results, we have considered the possibility that ATP efflux in our cells could be masked by the rapid catabolism caused at the extracellular side of the membrane by specific ectoenzymes (14, 15). The present article deals with the hypothesis that ATP is indeed released upon cell swelling and rapidly degraded to adenosine and that this nucleoside is the modulator of volume-sensitive anion channels, at least in our cell model.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The 9HTEo\(^-\) cell line was obtained by immortalization of human tracheal epithelial cells (16). Cells were grown at 37 °C in an atmosphere of 5% CO\(_2\) using a medium containing 45% Dulbecco’s modified Eagle’s medium, 45% Ham’s F-12, and 10% fetal clone II serum plus 100 units/ml penicillin, 100 \(\mu\)g/ml streptomycin, and 2 mM l-glutamine. Cells were plated on 35-mm Petri dishes at a density of 40,000/cm\(^2\) and cultured for 4 days before performing taurine and ATP transport studies.

**Solutions**—The isotonic standard solution used for efflux experiments contained 130 mM NaCl, 2 mM KCl, 1 mM KH\(_2\)PO\(_4\), 2 mM CaCl\(_2\), 2 mM MgCl\(_2\), 10 mM Na-Hepes pH 7.3, 10 glucose, and 20 mannitol (osmolality = 300 mosmol/kg). Hypotonic solutions (HS) were prepared by omitting mannitol and by lowering NaCl to 90 mM (205 mosmol/kg; 68% HS) or to 115 mM (250 mosmol/kg; 83% HS).

**Taurine and ATP Efflux**—For this study, we have taken advantage of...
the relatively large taurine permeability of VSOAC (11) to perform efflux experiments. This approach seems more appropriate than patch-clamp measurements to study the regulation of volume-sensitive channels, because taurine efflux experiments leave the cell interior undisturbed. The procedure for taurine efflux has been previously described (11). Briefly, cells were incubated for 1 h at 37 °C with 10 μM taurine and 0.2 μCi/ml [3H]taurine. At the end of incubation, the loading medium was discarded, and the cells were washed three times with ice-cold taurine-free medium. Taurine efflux experiments started with the addition of 1 ml of standard isotonic solution prewarmed at 37 °C. Every 5 min, for a total of 35 min, the efflux medium containing the taurine released by the cells was removed from the Petri dish and rapidly replaced by another ml of solution. The hypotonic shock was applied 15 min after the beginning of efflux. At the end of the experiment, cells were lysed by overnight incubation with 0.25 M NaOH. The radioactivity present in the efflux samples and in cell lysates was determined by liquid scintillation. Total incorporated taurine radioactivity was normalized for the total radioactivity accumulated in the NaOH extracts. Taurine remaining in the cells at a given time was determined by subtracting the radioactivity in the samples up to time t from that remaining in the NaOH extracts. The time course of taurine efflux was expressed by plotting fractional efflux (FE) versus time. The FE at a given time was calculated according to

\[
FE = \frac{[\text{radioactivity at time } t]}{[\text{radioactivity at time } 0]}
\]

where \( T_{\text{res}} \) is the amount of taurine released in a single efflux interval, and \( T_{\text{res}}/T_{\text{total}} \) is the amount of residual taurine remaining in the cells at the beginning of the interval.

For ATP release experiments, cells were loaded for 1 h with 2 μCi/ml [3H]AMP. After loading, the cells were treated with the same protocol of taurine efflux experiments except for the lysis, which was accomplished by three rapid freeze-thaw cycles. The radioactivity collected in the efflux samples was normalized for the total radioactivity accumulated into the cells as explained above. Except where indicated, isotonic and hypotonic solutions during the efflux always contained 1 μM diprydiamol to block the uptake of adenosine.

Ion Pairing Reverse-phase HPLC Analysis of [3H]-Labeled Nucleotides and Their Derivatives—Two hundred μl of cell supernatants or 100 μl of cell lysates were analyzed in an LKB liquid chromatograph (Amer sham Pharmacia Biotech) equipped with a Supelcosil LC-18-T column (15 cm x 4.6-mm inner diameter, 3-mm particle size; Supelco, Bellefonte, PA) and a Supelguard LC-18-T cartridge precolumn (Supelco). Buffer A was 60 mM KH2PO4, 5 mM tetrabutylammonium hydrogen sulfate, pH 6.0, containing 5% (v/v) methanol. Buffer B was 70% buffer A and 30% (v/v) methanol. The mobile phase was developed at a constant flow rate of 1.5 ml/min as follows: from 0 to 6 min, 0–60% buffer B; from 6 to 10 min, 60% buffer B; from 10 to 12 min, 60–0% buffer B. The system and column were reequilibrated in buffer A for an additional 8 min before the subsequent injection. The column eluate was monitored through a model Flow-one Beta radioactivity detector (Pack ard Instruments Co, Meriden, CT). Scintillation liquid was Ultima-Flo (Packard) at a flow rate of 3 ml/min, which yielded an approximately 1-min counting period. The radioactivity was monitored using an LKB multiwavelength UV detector. For ATP release experiments, cells were loaded for 1 h with 2 μCi/ml [3H]AMP. After loading, the cells were treated with the same protocol of taurine efflux experiments except for the lysis, which was accomplished by three rapid freeze-thaw cycles. The radioactivity collected in the efflux samples was normalized for the total radioactivity accumulated into the cells as explained above. Except where indicated, isotonic and hypotonic solutions during the efflux always contained 1 μM diprydiamol to block the uptake of adenosine.

RESULTS

According to our hypothesis, the hypotonic shock would cause a release of endogenous ATP. ATP would then be converted to adenosine by extracellular local catabolism. Adenosine, by interacting with adenosine receptors, would be responsible for the activation of VSOAC during the hypotonic shock. To test this model, we applied the hypotonic shock (68% HS) in combination with known adenosine receptor antagonists. DPCPX, which is a specific inhibitor of A1 receptors at low μM concentrations (17), significantly inhibited the peak of swelling-induced taurine release (Fig. 1, A and B). The effect was partial and dose-dependent with a maximal inhibition of 34% (Fig. 1B) and a half-effective concentration equal to 16 nM (Fig. 1B). Similarly, DMPX, an antagonist that acts at concentrations in the low μM range with a slight selectivity for A2 receptors versus A1 receptors (18), was also able to inhibit taurine efflux (Fig. 1, A and B). Maximal inhibition and half-effective concentration were 44% and 560 nM, respectively. When DMPX and DPCPX were used together at maximal concentrations (10 and 1 μM, respectively), the inhibition of taurine efflux was not different from that obtained by each antagonist individually. The inhibition of taurine efflux by adenosine receptor antagonists suggested that endogenous adenosine really contributes to swelling-dependent VSOAC activation. The next step was to assess the source of adenosine. Adenosine could directly originate from the cells or by hydrolysis of released ATP. Accordingly, we used αβMeADP, a potent blocker of 5’-ectonucleotidase, the ectoenzyme that hydrolyses AMP to adenosine (15, 19). αβMeADP inhibited in a dose-dependent fashion the taurine efflux elicited by the hypotonic shock (Fig. 2). Maximal effect was 37%, and half-effective concentration was 510 nM.

We previously showed that extracellular ATP application results in a strong potentiation of hypotonic shock effect (11). We asked whether exogenous ATP also acts through the conversion to adenosine. Therefore, we applied 100 μM ATP in the 68% HS with or without αβMeADP. Interestingly, αβMeADP strongly reduced the taurine efflux elicited by the combined stimulation with ATP and hypotonic shock (Fig. 3). Actually, αβMeADP inhibition seems to result from complete block of ATP effect in addition to the expected partial inhibition of the efflux induced by the hypotonic shock alone. Extracellular application of adenosine also potentiated swelling-induced taurine release as ATP (Fig. 3). Nevertheless, under these conditions, αβMeADP was completely ineffective.

[Image: 366x504 to 496x729]
Previous studies suggested that extracellular adenosine and ATP act in 9HTEo
2 and other airway cell lines by increasing intracellular Ca^{2+} through a phospholipase C (PLC)-based mechanism (20). If the hypotonic shock induced ATP and adenosine release, a PLC inhibitor should reduce the swelling-induced taurine efflux. We used U73122, an amino steroid that is able to potently block PLC when used at concentrations in the low μM range (21). U73122 (2 μM) reduced the peak of taurine efflux by 40% (Fig. 4A).

We reasoned that if adenosine were the endogenous agonist that mediates the effect of hypotonic shock on taurine channels, adenosine deaminase (ADA), which converts adenosine to inosine, should have an inhibitory effect. ADA caused a significant decrease of the swelling-induced taurine efflux, although smaller than that of αβMeADP, DPCPX, and DMPX (Fig. 4B).

Nucleosides are taken up by the cells through specific transporters, some of which are sensitive to inhibitors like nitrobenzylthioinosine (NBMPR) and dipyridamole (22, 23). We applied these compounds during the hypotonic shock to assess if they had an effect. Dipyridamole significantly increased taurine efflux under hyposmotic conditions (Fig. 5A). NBMPR was instead almost ineffective (Fig. 5B).

Previous experiments revealed that a mild hypotonic shock (83% HS) causes only a slight activation of the Cl^{−} channels. However, this effect can be strongly potentiated by Ca^{2+}-elevating agents such as ATP and ionomycin to obtain a channel activation comparable with that achieved with a stronger hypotonic shock (11). Fig. 6 shows that adenosine also acts by synergistically increasing the volume-sensitive taurine efflux. CGS21680, an agonist selective for A2A adenosine receptors (24), also increased taurine efflux (n = 3, not shown). Interestingly, αβMeADP did not inhibit adenosine effect but strongly antagonized that of ATP (Fig. 6).

We tested AMP-PNP because it has been often used as a nonhydrolyzable ATP analog. Accordingly, this compound had to be ineffective if ATP acts through the conversion to adenosine. Unexpectedly, AMP-PNP was active, although less than ATP, and its activity was strongly reduced by αβMeADP (Fig. 6).

Previous attempts to measure ATP release from 9HTEo-cells by the luciferin-luciferase assay were unsuccessful (11). In the present work, we have therefore utilized an HPLC-based approach. We reasoned that this method, by revealing the presence of ATP catabolites, would have allowed detection of ATP release even in the presence of a rapid extracellular hy-
hypotonic shock. We found that ionomycin elicited per se an increased radioactivity release from \(^{3}H\)AMP-loaded cells (Fig. 7B). Stimulation with ionomycin plus hypotonic shock (68% HS) induced a much higher efflux (Fig. 7B). Indeed, the effect was larger than the sum of the single responses to ionomycin and hypotonic shock.

HPLC analysis revealed that hypotonic shock, in the first 10 min of stimulation, caused the release of radioactive species whose elution times corresponded to those of adenosine, ADP, and ATP (Fig. 8, B and E). These peaks were absent in isotonic conditions (Fig. 8, A and C). On the other hand, supernatants of cells stimulated with ionomycin did not show a significant ATP, ADP, and adenosine release but a marked peak corresponding to inosine (Fig. 8D). When cells were treated with ionomycin plus the hypotonic shock (68% HS), supernatants had a higher content of ATP with respect to that observed with only the hypotonic shock (Fig. 8F; \(p < 0.05\)). Adenosine was also significantly increased (\(p < 0.01\)). Interestingly, when hypotonic shock (with or without ionomycin) was applied in the presence of \(\alpha\beta\)MeADP, the adenosine peak was strongly reduced, whereas AMP, which was always undetectable in the other experimental conditions, was clearly visible (Fig. 8, E and F).

Experiments performed using dipyridamole and ADA (Figs. 4 and 5) showed changes in VSOAC activity that we interpreted as due to changes in extracellular adenosine concentration. To validate this interpretation we removed dipyridamole from the efflux medium or, alternatively, we added exogenous ADA (0.2 units/ml). As expected, both procedures decreased the radioactive adenosine in the extracellular medium. Removal of dipyridamole reduced the adenosine peak from 1061 ± 133 to 585 ± 20 cpm (\(p < 0.05\); not shown). On the other hand, application of ADA completely abolished the adenosine peak and caused an increase of inosine from 128 ± 68 to 1401 ± 144 cpm (\(p < 0.01\)).

Our results suggest that 9HTEo− cells possess ectoenzymes able to degrade ATP. To directly demonstrate this process, cells were incubated for variable times with ATP (100 \(\mu\)M). The
supernatants were then analyzed by HPLC. After 1 h (not shown) and, more markedly, after 2 h (Fig. 9B), the peak corresponding to ATP decreased, and additional peaks appeared. These peaks corresponded to ADP, AMP, adenosine, and inosine. The process of ATP hydrolysis did not occur when ATP was incubated in the absence of cells. When αβMeADP was included in the extracellular solution, the adenosine peak was abolished, whereas that of AMP was significantly increased (not shown). AMP-PNP was able to activate VSOAC in a αβMeADP-sensitive way (Fig. 6). This result suggested that this compound can be converted to adenosine. Accordingly, we incubated the cells with this compound. HPLC analysis revealed the presence of a contaminant in the AMP-PNP stock solution (see Fig. 9C). According to the manufacturer instructions, this compound should be AMP-PNP after the loss of a phosphate moiety (i.e. ADP-NH₂). After incubation with 9HTEo- cells, the main and the secondary peaks were decreased, whereas a peak corresponding to adenosine was clearly evident (Fig. 9D). Incubation without the cells did not change the pattern of AMP-PNP-related peaks. Also in this case, αβMeADP decreased the adenosine peak and increased the AMP signal (not shown).

DISCUSSION

Cells are able to face a hypotonic shock by activating ion channels (VSOAC) permeable to Cl⁻ and organic osmolytes. The resulting efflux of osmolytes and water restores the original cell volume. The mechanisms responsible for VSOAC activation are unknown, although protein phosphorylation, membrane stretch, and changes in cytoskeletal organization have been proposed (25). Recently, it has been postulated that hypotonically induced ATP release represents an autocrine stimulus for VSOAC activation (12, 13). Our results indicate that the hypotonic shock elicits an ATP release also in 9HTEo-tracheal epithelial cells. ATP is then hydrolyzed to adenosine by specific ectoenzymes. However, in our cells, adenosine and not ATP is the agonist responsible for VSOAC activation. Several evidences support this scheme. First, taurine efflux is significantly reduced by adenosine receptor antagonists such as DPCPX and DMPX. These compounds act with half-effective concentrations, consistent with an inhibition at the level of adenosine receptors (17, 18). It is not clear which adenosine receptor is involved in the regulation of volume-sensitive Cl⁻ channels in 9HTEo- cells. The inhibition by DMPX and the activation by CGS21680 suggests the involvement of the A₂A type (24). However, DPCPX, a selective A₁ antagonist, has an unusually low IC₅₀ value for an A₂A receptor (24). Future studies with a series of agonists and antagonists could elucidate this point. The 5'-ectonucleotidase inhibitor αβMeADP, which blocks the conversion of AMP to adenosine (14, 15, 19), reduces the swelling-induced taurine release to an extent similar to adenosine receptor antagonists. A comparable reduction is also obtained by using the PLC inhibitor U73122. This result is consistent with the finding that exogenous adenosine stimulates PLC with consequent intracellular Ca²⁺ increase in airway epithelial cell lines (20). Our results are also consistent with previous patch-clamp studies in which we found that stimulation of adenosine receptors results in VSOAC activation (26).
Another indirect indication of adenosine involvement is the finding that nucleoside transporter inhibitors increase and that ADA, conversely, decreases taurine efflux. Such results can be explained by hypothesizing local variations of adenosine concentrations sensed by adenosine receptors. Indeed, dipyridamole would increase the availability of extracellular adenosine by blocking the reuptake. The stronger effect of dipyridamole compared with NBMPR can be explained by its ability to also inhibit NBMPR-insensitive transporters (22, 23). On the other hand, ADA probably decreases taurine efflux by degrading adenosine to inosine, thus lowering the adenosine concentration, which is sensed by adenosine receptors. ADA-dependent inhibition is smaller than that elicited by other treatments (DPCPX, DMPX, αβMeADP, U73122). This could be because of the difficulty encountered by a large enzyme to reach the sites on the plasma membrane where ATP release and hydrolysis occur or because the rate of adenosine production from ATP is faster than the rate of adenosine deamination to inosine.

To further verify our hypothesis, we applied extracellular ATP to assess whether it mimics the effect of the endogenous ATP. We found that ATP potentiates the effect of mild and strong hypotonic shocks (83% HS and 68% HS, respectively) and that this effect is completely blocked by αβMeADP. Such a result suggests that exogenous ATP is degraded to AMP by ectoATPase and ectoADPase and then to adenosine by 5′-ecto-nucleotidase. Adenosine then activates VSOAC by interacting with adenosine receptors. Indeed, the inhibition by αβMeADP can be bypassed by application of exogenous adenosine. In support of this model is the finding that extracellular UTP, which is often used as a hydrolysis-resistant ATP analog, activates taurine efflux. Furthermore, its effect can be blocked by αβMeADP. Our experiments, however, demonstrate that incubation of 9HTEo− with AMP-PNP results in the appearance of adenosine in the cell supernatant. This process is probably due to hydrolysis because it is abolished by αβMeADP. Our observation is consistent with recent findings that AMP-PNP can be actually hydrolyzed by extracellular ATPases (27).

Interestingly, other investigators have recently found that extracellular ATP may act in hypampal slices by rapid conversion to adenosine and stimulation of adenosine receptors (27). These authors have proposed a “channeling” model to explain the process. According to this model, the enzymes responsible for ATP degradation, and adenosine receptors are closely clustered. This organization ensures that the product of each reaction goes directly to the next enzyme in the cascade. This model, termed preferential substrate delivery, has been also proposed to explain the kinetics of extracellular ATP hydrolysis in endothelial cells (15). The close organization of ectoenzymes and receptors and the consequent channeling of the compounds through the degrading cascade would account for the poor effect of large molecules like ADA. It has been reported that ectonucleotidases are clustered in caveolae, i.e., small invaginations of plasma membrane (28). This organization could also contribute to confine ATP and its catabolites so that ectoenzymes and purinergic receptors would sense local and not bulk concentrations. It is interesting to observe that our results clarify previously unresolved observations done with patch-clamp experiments on 9HTEo− cells (26). Actually, we found that the activation of volume-sensitive Cl− channels by extracellular ATP could be inhibited by an adenosine receptor antagonist (DMPX). We may now conclude that this surprising behavior was because of the hydrolysis of ATP to adenosine, which is the actual agonist. Such a conclusion reveals that the mechanism of ATP hydrolysis by ectoenzymes might be relevant even during patch-clamp experiments in which cells are continuously perfused with fresh solution.

We have demonstrated by an HPLC-based approach that ATP is indeed released during the hypotonic shock (68% HS) in 9HTEo− tracheal epithelial cells. Previous attempts to detect ATP by the luciferase assay were unsuccessful (11). We have no explanation for these previous negative results. It is possible that rapid nucleotide hydrolysis and the mechanism of preferential substrate delivery might reduce the amount of ATP that diffuses from the cells into the bulk solution.

Interestingly, the ATP release does not occur when the milder hypotonic shock (83% HS) is applied. This behavior explains, at least in part, the quite different channel activity observed with the two hypotonic solutions. Indeed, with 68% HS, the taurine efflux is so fast that more than 50% of intracellular taurine is released in the first 5 min of stimulation (Fig. 1). On the contrary, with 83% HS, only 3% of taurine is released (Fig. 6). In other words, the relationship between the efflux and the extracellular osmolality is not linear, but a threshold for activation exists below 250 mosmol/kg. This threshold is probably because of the activation of ATP release only with strong hypotonic shocks. Actually, when exogenous adenosine is provided under mild hypotonic conditions (Fig. 6), the taurine efflux increases to levels comparable with those attained with 68% HS.

Intracellular Ca2+ increase by application of ionomycin in isotonic conditions did not result in ATP efflux but caused a large release of inosine. However, combined stimulation with hypotonic solution (68% HS) and ionomycin elicited a stronger release of ATP if compared with hypotonic shock alone. In parallel, extracellular adenosine was also increased. αβMeADP markedly decreased adenosine and caused the appearance of AMP in hypotonic conditions. This behavior suggests that adenosine is not directly released by the cells but probably arises from ATP hydrolysis. Inosine was not lowered by αβMeADP, thus indicating that it does not derive from adenosine. Actually, the effect of ionomycin in isotonic conditions suggests that there is a separate Ca2+-dependent mechanism for inosine release.

Our results do not match exactly those of other investigators (12). First of all, we find that adenosine and not ATP is the autocrine agonist of VSOAC during the hypotonic shock. Another difference lies in the degree of channel activation, which can be accounted for by the autocrine signaling. Actually, Wang et al. (12) reported that treating hepatoma cells with ATP-degrading enzymes like apyrase completely blocks the swelling-induced chloride channel activation. In our experiments, the various treatments only partially inhibited the effect of the stronger hypotonic shock. It appears therefore that in 9HTEo− cells, adenosine and ATP release are not essential for swelling-induced activation of VSOAC. However, the release of these substances appears to play an important modulatory role. Our findings suggest that important tissue-specific differences might exist in the mechanism of VSOAC activation.

In conclusion, our experiments reveal that hypotonic shock induces a release of ATP from airway epithelial cells. ATP is then converted on the extracellular side of the membrane to adenosine, which binds to adenosine receptors to increase the activity of volume-sensitive chloride channels. This autocrine mechanism, which probably affects the speed and extent of regulatory volume decrease (29), is likely modulated by intracellular Ca2+. The regulation of ATP release by cell volume and Ca2+ is probably important in the process of transepithelial chloride transport. Indeed, extracellular ATP represents a strong stimulus for chloride secretion in airway epithelia (30). Several agonists, including ATP, induce an intracellular Ca2+...
increase in airway epithelial cells (30–32). This response could elicit intracellular ATP release, which would further stimulate the cells in an autocrine/paracrine fashion. The sensitivity of the ATP release process to cell volume could help to control the activation of chloride channels. Indeed, excessive Cl\(^{-}\) channel activity and Cl\(^{-}\) secretion would cause cell shrinkage. This should stop the release of ATP, which otherwise would continue to stimulate Cl\(^{-}\) channels.

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