Stimulation of chloride secretion by P1 purinoceptor agonists in cystic fibrosis phenotype airway epithelial cell line CFPEo-

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1 P1 purinoceptor agonists like adenosine have been shown to stimulate Cl− transport in secretory epithelia. In the present study, we investigated whether P1 agonist-induced Cl− secretion is preserved in cystic fibrosis airway epithelium and which signalling mechanism is involved. The effects of purinoceptor agonists on Cl− secretion were examined in a transformed cystic fibrosis airway phenotype epithelial cell line, CFPEo-.

2 Addition of adenosine (ADO; 0.1–1 mM) markedly increased 125I efflux rate. The rank order of potency of purinoceptor agonists in stimulating 125I efflux was ADO > AMP > ADP ≈ ATP. A similar order of potency was seen in transformed cystic fibrosis nasal polyp cells, CFNPEo- (ADO > ATP > AMP > ADP). These results are consistent with the activation of Cl− secretion via a P1 purinoceptor.

3 The P1 agonists tested (at 0.01 and 0.1 mM) revealed a rank order of potency of 5′-N-ethylcarboxamide adenosine (NECA) > 2-chloro-adenosine (2-Cl-ADO) > R-phenylisopropyl adenosine (R-PIA).

4 The known potent A2 purinoceptor agonist (A2AR) agonist, 5′-(N-cyclopentyl) carboxamidoadenosine (CPCA, 2 µM) but not the A1 adenosine receptor agonist, N6-phenyl adenosine (N6-phenyl ADO, 10 µM) markedly increased 125I efflux rate (baseline, 5.9 ± 2.0% min−1, + CPCA, 10.9 ± 0.6% min−1; P < 0.01). The stimulant effect of CPCA (10 µM) was abolished by addition of the A2AR antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) (100 µM; reported KI = 11 ± 3 µM). These results favour the involvement of A2AR.

5 ADO (0.1–1 mM) and CPCA (2 µM) both induced a marked increase in intracellular [Ca2+] ([Ca2+]; the effect of the latter was again abolished by pretreatment of the cells with DMPX. By contrast, N6-phenyl ADO did not affect [Ca2+].

6 In patch-clamp experiments, ADO (1 mM) induced an outwardly-rectified whole-cell Cl− current (baseline, 2.5 ± 0.8 pA pF−1, + ADO, 78.4 ± 23.8 pA pF−1; P < 0.02), which was largely inhibited in cells internally perfused with a selective inhibitory peptide of the multifunctional Ca2+/calmodulin-dependent protein kinase, CaMK [273-302] (20 µM), as compared to a control peptide, CaMK [284-302]. Addition of BAPTA (10 mM), a Ca2+ chelator, to the perfusion pipette also abolished the ADO-elicted Cl− current.

7 In conclusion, our results suggest that A2AR participates in regulation of airway Cl− secretion via a Ca2+-dependent signalling pathway, which involves CaMK and appears to be at least partially conserved in cystic fibrosis airway epithelial cells.

Keywords: P1 purinoceptor; cystic fibrosis; airway epithelium; chloride channel; Ca2+; multifunctional calcium/calmodulin-dependent protein kinase

Introduction

Cystic fibrosis (CF) is the most common inherited fatal disease among Caucasians, with an estimated incidence of 1 in 2000 to 3000 newborns (Boat et al., 1989). The primary physiological defect associated with CF is altered salt and water transport in epithelial cells (Boat et al., 1989; Anderson et al., 1992) which is normally regulated by an adenosine 3′:5′-cyclic monophosphate (cyclic AMP) dependent pathway. Considerable efforts have been made in recent years to improve epithelial Cl− secretion through alternate signal-transduction pathways that are independent of cyclic AMP. P1 purinoceptor agonists, ATP and UTP, have recently been shown to enhance Cl− secretion in CF nasal epithelial cells (Mason et al., 1991; Stutts et al., 1992), and have been tested in clinical trials (Knowles et al., 1991). Other purine analogues, namely P1 purinoceptor agonists like adenosine (ADO), have also been shown to stimulate Cl− transport in secretory epithelia (Pratt et al., 1986; Barrett et al., 1990) including airway cells. We questioned whether P1 purinoceptor-mediated Cl− secretion is preserved in CF airway epithelial cells, and, if so, what signalling mechanism is involved. We investigated the effects of various P1 purinoceptor agonists in a simian virus 40 (SV40)-transformed CF phenotype airway epithelial cell line, CFPEo- (Cozens et al., 1991; 1992).

Purinoceptors have been classified after Burnstock (1990) as P1 and P2 subtypes, based on their preferences for ADO or adenosine nucleotides. A P1 receptor typically responds to these agents in the rank order of potency of ADO > AMP > ADP > ATP and is therefore also named adenosine receptor; the responsiveness of the P1 subtype usually shows the reverse order (Stiles, 1991; Olah & Stiles, 1992). P1 receptors are further subclassified into A1 and A2 adenosine receptor subtypes. A1 adenosine receptor (A1AR) generally responds to ADO analogues in the potency order of R-phenylisopropyl adenosine (R-PIA) > 2-chloro-adenosine (2-Cl-ADO) > 5′-N-ethylcarboxamide adenosine (NECA) (Ukena et al., 1987; Williams, 1987); whereas A2AR responds in the order of NECA > 2-Cl-ADO > R-PIA (Stiles, 1991). A2AR has been shown to couple to a variety of effector systems, including

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adenylate cyclase, guanylate cyclase, membrane ion channels, and phospholipases (Olsson & Pearson, 1990). So far, the only established effector system for A2AR is adenylate cyclase (Stiles, 1991). In this study, we demonstrate that P2 purinoceptor agonists act on A2AR to activate airway Cl− secretion via a Ca2+-dependent signalling pathway, which appears to be preserved in CF airway epithelial cells.

Methods

Cell culture

CFPEno- cells were transformed as described (Cox et al., 1991; 1992). Cells were grown in Minimum Essential Medium supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NY, U.S.A.), 100 μml−1 penicillin and 0.2 mg ml−1 streptomycin (Biofluids, Rockville, MD, U.S.A.). The culture medium was replenished 2–3 times per week. Cells were used at passage 63–70.

125I efflux assay

Cells were plated on 22 × 22-mm square plastic coverslips (VWR, San Francisco, CA, U.S.A.) and used at 90–100% confluency ∼1 week after seeding. The efflux solution contained (in mM): 135 NaCl, 1.2 CaCl2, 1.2 MgCl2, 2.4 K2PO4, 0.6 K2HPO4, 10 glucose, and 10 HEPES (pH 7.4). In high-K+ (120 mM) efflux solution, 114.6 mM KCl substituted for equal amount of NaCl. Cells were first loaded with 20 μCi ml−1 125I for ∼2 h in a water bath gassed with 100% O2 at 37°C. Extracellular 125I was eliminated by rapidly rinsing the cell monolayer on coverslip three times in efflux solution for a cumulative time of 1 min. The efflux experiment was then carried out by sequentially transferring the cell monolayer/ coverslip into appropriate time P2 vehicle solutions (usually 1 min), through a series of cell culture dishes (Costar, Cambridge, MA, U.S.A.) containing 3 ml efflux solution plus the desired agent(s) at room temperature. The cell monolayer/coverslip was retained in the last culture dish of transfer. 125I effused into each dish was counted individually in a gamma radiation counter (LKB, Gaithersburg, MD, U.S.A.). Non-efflux counts were determined by counting the cells together with the 3 ml efflux solution in the last cell culture dish.

Fluorescence measurement of [Ca2+]i

CFPEno- cells were plated on 9 × 22-mm rectangular glass coverslips (Wheaton, Millville, NJ, U.S.A.) and studied at 90–100% confluency ∼1 week after seeding. Cells were incubated in efflux solution containing 5–10 μM fura-2 AM and 0.05% (w/v) pluronic F127 for 15–20 min at 37°C and rinsed with dye-free solution. The coverslip was then mounted vertically in an acrylic cuvette at an angle of ∼60 degrees from the incident light. Agents were added to the cuvette during an experiment by means of a Hamilton syringe. Fluorescence was measured in a spectrophotometer (SLM-AMINCO, Urbana, IL, U.S.A.) at 30°C. Excitation wavelength was altered between 340 and 380 nm every 0.2 s and the emission fluorescence monitored at 510 ± 10 nm [Ca2+]i was determined as described by Grynkiewicz et al. (1985).

Electrophysiology

Whole-cell patch-clamp experiments were performed in single CFPEno- cells grown on glass coverslips 1–2 days following seeding. Cells/coverslip were placed in a 1 ml acrylic chamber on the stage of a Zeiss IM inverted microscope and bathed in a solution containing (in mM) Tris-Cl 170, MgCl2 1, CaCl2 2.5, glucose 15, and HEPES 10 (pH 7.4; ∼330 mOsm kg−1), at 25–30°C. The pipette solution contained (in mM): CsCl 140, MgCl2 2, EGTA 1, MgATP 2, glucose 10 and HEPES 5 (pH 7.35; ∼300 mOsm kg−1). The bath solution was made ∼30 mOsm kg−1 hypertonic compared with the pipette solution to prevent hyponicity-induced Cl− current (Worrell et al., 1989). Micropipettes were made as described by Hamill et al. (1981) and had a tip resistance of 2–3 MΩ. Whole-cell currents were recorded with an Axopatch amplifier (Axon Instruments, Foster City, CA, U.S.A.). Voltage-clamp protocols were run with an aid of a Tencar 12-bit A/D-D/A converter and an IBM-AT computer. Cells were filtered at 1 kHz, were displayed on a strip-chart recorder and stored in floppy disks. Data were analyzed by means of pClamp, version 5.5 (Axon Instruments).

Materials

CPCA, DMPX and N-phenyladenosine were purchased from Research Biochemical Inc. (Nalick, MA, U.S.A.). Fura-2-acetoxymethyl ester (fura-2-AM), 1,2-bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid, tetracesium salt (BAPTA) and pluronic F127 were from Molecular Probes (Eugene, OR, U.S.A.). Other reagents were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Synthetic peptides CaMK [273-302] and CaMK [284-302] were kindly provided by Dr Howard Schulman, Stanford University.

Results

Effect of purinoceptor agonists on 125I efflux rate

We initially studied and compared the effect of adenine nucleotides ATP, ADP, and 5'-AMP on 125I efflux rate in CFPEno- cells to test whether the P2 purinoceptor plays a predominant role in regulating membrane conductive Cl− transport. We concentrated on CF phenotype airway cells since P2 purinoceptor agonists are in clinical trial as a therapeutic agent to improve airway function in CF, presumably by enhancing Cl− secretion. 125I was used in the efflux assay because iodine is selectively transported through the Cl−-conductive pathway by epithelial cells (Widdicombe & Welsh, 1980). As illustrated in Figure 1a, 125I efflux rate
was stimulated differentially by adenine nucleotides following the rank order of potency of 5'-AMP > ADP > ATP. In order to rule out the possibility that the increased 125I efflux rate is secondary to an activated K+ conductance, which hyperpolarizes the cell membrane and increases the driving force for Cl- conductive exit, the experiment was repeated in a high-K+ (120 mM) bathing medium. Under these conditions, an identical order of stimulation by the agonists was observed (Figure 1b). We have also examined and compared the effect of ADO and adenine nucleotides in a cell line transformed from CF nasal polyps, CFNPEo- (Cozens et al., 1992); these agents (1 mM) stimulated 125I efflux following a similar order of potency (ADO ≫ ATP > AMP > ADP; data not shown). As summarized in Figure 2, ADO and adenine nucleotides augmented 125I efflux in CFPEo- cells in the order of potency of ADO > AMP > ADP ≈ ATP, both at the dose of 1 mM and 0.1 mM. ADO showed much less effect when given at a lower dose; in a set of 'paired' experiments performed under identical conditions on the same day, additions of 0.01, 0.1 and 1 mM ADO increased 125I efflux rate by 0.2, 1.4, and 4.1% min⁻¹, respectively. These results are consistent with activation of Cl- conductance via a P1 purinoceptor. To decipher the subtype of P1 purinoceptors involved in purine analogue-induced Cl- secretion, the effects of P1 selective purinoceptor agonists were subsequently examined and compared at 10 and 100 μM, respectively. As shown in Figure 1c and Figure 2, among the P1 agonists tested, 125I efflux rate was stimulated in the order of NECA > 2-Cl-ADO > R-PIA. CPCA is a potent A2AR agonist (Bruns et al., 1986; Daly, 1982). We have tested the effect of CPCA (2 μM) with that of a selective A2AR agonist, N6-phenyl ADO (10 μM; Daly et al., 1986). As shown in Figure 1d and Figure 2, 125I efflux rate was potently stimulated by CPCA but was only slightly stimulated, if at all, by N6-phenyl ADO. These results favour the involvement of the A2AR subtype.

**Effect of P1 purinoceptor agonists on [Ca2+]i.**

CF is characterized by defective regulation of the CFTR Cl- channel by cyclic AMP-dependent protein kinase and protein kinase C (Boat et al., 1989; Hwang et al., 1989; Anderson et al., 1992). In CF airway however, Cl- secretion remains inducible by raising [Ca2+]i (Wagner et al., 1991; 1992; Chan et al., 1992). We have tested whether P1 purinoceptor agonists stimulate Cl- secretion in CFPEo- cells by mobilizing intracellular Ca2+. Application of ADO, at 1 and 0.1 mM, caused a marked transient increase in [Ca2+]i (Figure 3a and b). The effect of ADO was much less pronounced when given at a lower dose (not shown). In concert with the 125I efflux data (see Figure 1d), CPCA but not N6-phenyl ADO induced elevation of [Ca2+]i (Figure 3c and d). The effect of P1 purinoceptor agonists on [Ca2+]i, is summarized in Table 1.

A2AR antagonist DMPX abolishes stimulation by CPCA

3,7-Dimethyl-1-propargyloxanthine (DMPX) is a potent and selective A2AR antagonist (Seale et al., 1988). We have examined the effect of DMPX on CFPEo- cells using intracellular Ca2+ mobilization and CFPEo- stimulated 125I efflux. DMPX was used at 100 μM, ~9 times the reported Ki value of 11 ± 3 μM (Seale et al., 1988); the dose of DMPX was increased to 10 μM. Pretreatment of cells with DMPX (6–10 min) abolished the stimulatory effect of CPCA both on [Ca2+]i (Figure 4a) and on 125I efflux (Figure 4b). These results suggest that P1 purinoceptor agonists act mainly on A2AR to stimulate Cl- secretion, which is mediated through a Ca2+-dependent pathway.

**Activation of membrane Cl- channels by P1 purinoceptor agonists: role of CaMK**

Bath application of ADO (1 mM) induced an outwardly-rectifying whole-cell current characteristic of Ca2+-stimulated membrane Cl- current (Figure 5a). The recorded current should be predominantly due to flow of Cl- ions because Na+ and K+ were omitted in the bath and pipette solutions and Ca2+, used to replace pipette K+, is known to block K+ channels. The reversal potential was near 0 mV (Figure 5b), in good agreement with the predicted Nernst potential for Cl- (~5 mV).

Ca2+/calmodulin-dependent protein kinase (CaMK) has been shown to mediate Ca2+-stimulated Cl- currents in secretory epithelia (Worrell & Frizzell, 1991; Chan et al., 1992; Wagner et al., 1992) and human T lymphocytes (Nishimoto et al., 1991). The Ca2+-dependent pathway
Table 1  Effects of addition of P1 purinoceptor agonists on [Ca^{2+}]i (nM) in CFPEo-
cells

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<th>Baseline</th>
<th>+ Agent</th>
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<tr>
<td>ADO (1 mM)</td>
<td>67 ± 23</td>
<td>324 ± 46</td>
<td>257 ± 33*</td>
<td>3</td>
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<tr>
<td>ADO (0.1 mM)</td>
<td>61 ± 8</td>
<td>277 ± 44</td>
<td>216 ± 43*</td>
<td>5</td>
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<tr>
<td>N^6-phenyl ADO (10 μM)</td>
<td>102 ± 13</td>
<td>108 ± 12</td>
<td>7 ± 3</td>
<td>4</td>
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<tr>
<td>CPCa (2 μM)</td>
<td>59 ± 5</td>
<td>252 ± 31</td>
<td>193 ± 34*</td>
<td>3</td>
</tr>
<tr>
<td>CPCa (10 μM) in presence of DMPX (100 μM)*</td>
<td>119 ± 23</td>
<td>127 ± 25</td>
<td>8 ± 2</td>
<td>3</td>
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Data are presented as mean ± s.e.mean. n, number of cell monolayers studied. *Significantly different from the baseline [Ca^{2+}]i, (P<0.05) by Student’s paired t test. †CPCA was added ~8 min after the addition of DMPX.

Figure 4  Stimulation of CPCa is abolished by A_{2}AR antagonist, DMPX. (a) Effect of CPCa (10 μM) on [Ca^{2+}]i in presence of DMPX (100 μM). The time-break shown was ~3 min. (b) Effect of CPCa on 18F efflux in the presence (●) and absence (●) on DMPX. n = 4 each.

Involves CaMK-mediated phosphorylation of a Cl^− channel/transport protein entirely separate from CFTR, since antisense depletion of CFTR protein eliminates cyclic AMP-dependent but not Ca^{2+}-dependent Cl^− secretion (Wagner et al., 1992). As P1 purinoceptor agonist-stimulated Cl^− secretion correlated with intracellular Ca^{2+} mobilization, we next examined whether CaMK is involved in the signal transduction pathway. A selective inhibitory peptide of CaMK (a synthetic peptide containing the autoinhibitory region of CaMK), CaMK [273-302], which inhibits brain CaMK activity at 1 μM (Malinow et al., 1989), and a control peptide, CaMK [284-302], which is a truncated version of CaMK [273-302] and does not inhibit CaMK activity (Malinow et al., 1989), were added separately into the cytosol of different groups of CFPEo-cells, through the patch-clamp pipette. As shown in Figure 6, ADO-stimulated Cl^− currents were largely inhibited by the inhibitory peptide CaMK [273-302] (P<0.01) but not by the control peptide CaMK [284-302]. In concert, incorporation of BAPTA (10 mM), a Ca^2+ chelator, to the pipette solution, to prevent intracellular Ca^[2+], elevation and activation of CaMK, also abolished ADO-induced whole-cell Cl^− currents (baseline 2.5 ± 0.5 pA pF^-1; + ADO, 2.3 ± 0.3 pA pF^-1; n = 3). These results strongly suggest that the action of P1 purinoceptor agonists is mediated via a Ca^{2+}-dependent signalling pathway involving CaMK.

Figure 5  Effect of ADO on membrane Cl^− current. (a) Whole-cell Cl^− current recorded at baseline (above) and after stimulation (below) of ADO (1 μM). Dotted line indicates zero-level Cl^− current. The resting membrane potential was held at −70 mV. To examine the current-voltage (I-V) relation of a recorded current, membrane potential was sequentially altered from −100 to +100 mV in 50 mV steps, using the voltage-clamp protocol given in the inset. Data were averaged over the final 50 ms of the current pulses. (b) I-V relations of Cl^− currents recorded at baseline (●) and after stimulation (●) of ADO. Data were normalized by dividing by cell capacitance, an index of cell surface area (Wagner et al., 1991), and presented as mean ± s.e.mean (n = 7 cells).

Discussion

ADO and adenine nucleotide induced Cl^− (I^−) conductive loss in the CF airway epithelial cell lines, CFPEo- and CFNPEo-. In both cases, ADO was the most potent stimulant among the four purine analogues tested. Thus, under our experimental conditions, the P1 purinoceptor agonist, ADO was more effective in stimulating Cl^− secretion than the P2 agonist, ATP. These results suggest that the purinoceptor agonists-stimulated Cl^− secretion is mainly
mediated by the P1 subtype. P1 purinoceptor agonists differentially stimulated Cl− secretion in CFPEo- cells in the rank order of potency of NBME > 2-Cl-ADO > R-PIA. These results are consistent with the involvement of A2AR. A higher dose of these agents and ADO was needed to induce Cl− secretion, compared with that needed in the native canine airway epithelial cells (Pratt et al., 1986). ADO 0.01 mM was not sufficient to increase substantially 32I efflux in CFPEo- (see Results). This is consistent with our previous observation (Coesens et al., 1991) that transformed epithelial cells, after being passed a number of times, tend to show reduced sensitivities to various Cl− secretagogues. In addition, the higher dose of P1 agonists needed to induce Cl− secretion in CFPEo- may partly be due to an attenuated response of CF airway epithelia to Ca2+-mediated stimulation. Ca2+ ionophore A23187 was shown previously to enhance Cl− transport in CF airway epithelial cells to a level of ~60% that of normal cells (Frizzell et al., 1986; Boucher et al., 1989).

What is the signal transduction mechanism involved in A2AR-mediated Cl− secretion in CFPEo-? We have shown recently that cyclic AMP does not affect membrane Cl− channel activities in transformed CF airway epithelial cells (Wagner et al., 1991). The effect of ADO and A2AR agonist CPA on [Ca2+]i, and the abolition of CPA-induced increase in [Ca2+]i by the A2AR antagonist DMPX, implicate the involvement of a Ca2+-dependent pathway. The transient increase in [Ca2+]i brought about by ADO and CPA may result from the stimulation of phospholipase C and phosphatidylinositol turnover. A2AR agonists have recently in a rat tumour mast cell that NECA induced transient elevation of cytosolic inositol phosphates as well as [Ca2+]i, accompanied by a secretory response to P1 purinoceptor agonists in a rank order of potency implying a role of the A2AR. Our patch-clamp experiments show that CaMK also participates in ADO-stimulated Cl− secretion, as previously studied for the calcium release induced by paired stimuli (Wagner et al., 1991; Worrell & Frizzell, 1991). The increase in [Ca2+]i elicited by ADO or CPA may cause autophosphorylation of CaMK, rendering it active even after [Ca2+]i returns towards the baseline level (MacNicol et al., 1990). These results demonstrate that the effect of ADO-induced elevation of [Ca2+]i is further transmitted by CaMK. This implies that A2AR may couple to effector systems other than adenylate cyclase, which is the only reported one thus far (Stiles, 1991).

How do these findings compare to previously published reports? The exact nature of the purinoceptor that is linked to ion transport in secretory epithelia is unclear from the cumulative published literature. Several previous published studies, most notably of canine tracheal epithelium (Pratt et al., 1986) and human T4 colonic carcinoma cells (Barrett et al., 1990), demonstrated ADO-induced Cl− secretion mediated through an A2AR, as indicated by the rank order of potency of ADO analogues. In each of these studies, A2AR appeared to be coupled to adenylyl cyclase and generation of cyclic AMP, though in the latter study (Barrett et al., 1990), ion transport was stimulated at concentrations below that required to increase cyclic AMP. Barrett et al. (1990) further showed that ADO did not affect [Ca2+]i in T4 cells and initiated a phosphorylation profile consistent with cyclic AMP- rather than Ca2+-dependent kinase. If ADO-induced Cl− secretion is cyclic AMP-mediated, it presumably occurs via CFTR and therefore should be defective in CF- derived cells. Our studies indicate, however, that A2AR is coupled to Ca2+ and is preserved in CF epithelial cells, as expected for Ca2+-dependent Cl− secretion (Wagner et al., 1991).

In contrast to our findings and those cited above, Boucher and his colleagues demonstrated that extracellular nucleotides regulated ion transport across human airway epithelium, both in vivo (Knowles et al., 1991) and in freshly excised intact tissue (Mason et al., 1991), by means of a P2 receptor. The potency order of nucleotide analogues and responsiveness to UTP suggested the presence of one or more P2 receptor subtypes distinct from the previously characterized P2x and P2y subtypes (Burnstock & Kennedy, 1985). ADO was relatively ineffective in mediating ion transport. In this case, nucleotide-induced ion transport changes were linked to changes in [Ca2+]i, and were preserved in CF airway epithelium. Nucleotide-induced changes in short-circuit current were attributed to increments in the rate of Na+ absorption (Barrett et al., 1990) rather than primarily through changes in Cl− secretion. Primary changes in Na+ absorption could not explain, however, our whole-cell patch-clamp studies of CaMK-mediated outwardly rectifying Cl− currents. Differences in the findings with respect to the purinoceptor subtype andionic mechanism may be partially attributable to studies of relatively intact human alveolar epithelial cells (Barrett et al., 1990) vs. studies of the transformed airway epithelial cells. In the relatively more intact tissue preparation, metabolism of ADO may mask ADO-induced changes in Cl− secretion. In support of this, it has been shown that ADO is relatively impotent in causing Cl− secretion in rabbit ileal mucosa because of uptake and metabolism (Dobinson et al., 1990).

We have shown that P1 purinoceptor agonists can stimulate Cl− secretion in CF airway epithelial cells via an alternate Ca2+-dependent signalling pathway, thus suggesting a potential means of circumventing the CF defect. In isolated cells, the P1 purinoceptor agonists appeared to be con-

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**Figure 6** ADO activates Cl− secretion via CaMK. (a) Effect of ADO (1 mM) on whole-cell Cl− current in the presence of CaMK control peptide (CaMK [284-302]) left) and in the presence of CaMK inhibitory peptide (CaMK [273-302]; right), respectively. Cell was internally perfused with the peptide (20 µM) by addition to the pipette solution. Shown are whole-cell Cl− current recorded at baseline (above) and after stimulation (below) of ADO. (b) Baseline outward Cl− current (open columns) and maximal outward Cl− current induced by ADO (solid columns) in absence of peptide (No peptide, n = 7), in the presence of CaMK [284-302] (CaMK control; n = 5), and in the presence of CaMK [273-302] (CaMK inhibitor; n = 5), respectively. **P < 0.01 compared with ADO-activated Cl− current in the absence of peptide (No peptide) by Student’s unpaired t test.
considerably more potent in inducing Cl⁻ secretion than the P₁ agonists like ATP. Thus, nonmetabolizable P₁ purinergic agonists might be useful therapeutically in treatment of CF. It should be noted, however, that while ADO does not induce bronchoconstriction in normal subjects, it enhances bronchoconstriction in asthmatic patients (Cushley et al., 1984). This side-effect of ADO can be attenuated by nedocromil sodium and sodium cromoglicate (Crimi et al., 1986).

In conclusion, our results suggest that P₁ purinergic agonists act via A₁AR to stimulate Cl⁻ secretion in airway epithelial cells. The stimulatory effect of P₁ agonists is mediated via a Ca²⁺-dependent signalling pathway, which appears to be, at least partially, preserved in CF cells.

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