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ATP and A_1 Adenosine Receptor Agonists Mobilize Intracellular Calcium and Activate K^+ and Cl^- Currents in Normal and Cystic Fibrosis Airway Epithelial Cells*

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The effect of purinergic compounds on $[Ca^{2+}]_i$ and membrane currents of cell lines derived from the airway epithelium of normal and cystic fibrosis individuals has been investigated.

2-Chloroadenosine (2-CADO), as well as other agonists of the A_1 adenosine receptors, causes a transient elevation of cytosolic $[Ca^{2+}]$ that is antagonized by the A_1 adenosine receptor antagonist 8-cyclopentyl-1,3 dipropylxanthine (DPCPX). ATP is also effective, but at a lower extent. The $[Ca^{2+}]_i$ increase induced by 2-CADO and ATP is abolished by preincubation with phorbol 12-myristate 13-acetate and the Ca^{2+}-ATPase inhibitor thapsigargin. This latter result suggests that purinergic agonists mobilize Ca^{2+} from inositol 1,4,5-trisphosphate-sensitive stores. Pertussis toxin completely inhibits the effect of 2-CADO, whereas only it partially affects that of ATP, suggesting the involvement of different types of G proteins. Perforated patch clamp experiments carried out in both current clamp and voltage clamp modes show that 2-CADO and ATP activate K^+- and Cl^--selective membrane currents, with a mechanism inhibited by preincubation with DPCPX and thapsigargin. These data indicate that activation of adenosine A_1 receptor, in a similar way to ATP receptor, causes $[Ca^{2+}]_i$ increase and ion channels activation through a transduction mechanism that is not impaired in cystic fibrosis airway epithelial cells.

In cystic fibrosis (CF),[1] the most common autosomal recessive disease among Europeans, defective regulation of a cAMP-dependent Cl^- channel reduces Cl^- ion secretion in epithelial cells (for a review, see Ref. 1). Regulation of Cl^- channel by intracellular Ca^{2+} appears intact in CF epithelia (2).

An increasing interest has been recently devoted toward the purinergic regulation of epithelial membrane currents. It has been shown that extracellular ATP can stimulate Cl^- secretion in both normal and CF airway epithelial cells (3, 4) and that this effect is associated with the elevation of intracellular $[Ca^{2+}]_i$ (4) and of inositol phosphates (5).

Adenosine and adenine nucleotides have been involved with extracellular signaling by interacting with specific membrane receptors: P_1 receptors are activated by adenosine and P_2 receptors are activated by ATP (6, 7).

P_1 receptors were originally classified into two major subtypes, A_1 and A_2, depending on whether their activation stimulates (A_2) or inhibits (A_1) adenylate cyclase activity. More recently their classification has been based on the order of agonists and antagonists potency in receptor binding studies (8). There is now data that suggests that adenosine receptors might be coupled to a transduction system different from adenylate cyclase, involving phospholipase C activation via pertussis toxin-sensitive G proteins (9, 10).

P_2 receptors have been tentatively divided into four subtypes: P_{2x}, P_{2y}, P_{2z}, and P_{2t} (11). The P_{2y} receptors appear to stimulate phospholipase C, via GTP-binding proteins (12).

In a previous work, we reported that, in addition to ATP, an adenosine analog, 2-chloroadenosine (2-CADO), was able to produce an increase of the intracellular free $[Ca^{2+}]$ in a cell line (9HTEo⁻) derived from the human tracheal epithelium (13). We also showed that both compounds were able to induce a sustained activity of volume-sensitive Cl^- currents (13).

To further define the physiological relevance of such an observation, we have extended our study to other airway epithelial cells derived from CF patients. As in 9HTEo⁻, CFNPE9o⁻ (14) and CFPEo⁻ cells also showed increases in cytosolic free $[Ca^{2+}]$ after extracellular addition of either ATP or adenosine analogs. Furthermore, these purinergic compounds were able to activate K^+ and Cl^- currents in CF cells, as revealed by either current clamp and voltage clamp recordings. Similar results were also obtained with 56FHTEo⁻ cells (15) derived from a non-CF fetal trachea.

EXPERIMENTAL PROCEDURES

Cell Cultures—9HTEo⁻ and 56FHTE8o⁻ were established by transformation of normal adult and fetus tracheal epithelial cells, respectively (15, 16). CFNPE9o⁻ (14) and CFPEo⁻ were obtained from nasal polyps and the submucosal glands of two different CF patients, respectively. Cells were cultured as described in Ref. 17.

Measurement of $[Ca^{2+}]_i$—Loading with fura-2 was performed essentially as described in Ref. 13. Briefly, cells were trypsinized and incubated (2×10^6/ml) for 30 min at 37 °C with continuous stirring in HAMs' F-12 growth medium supplemented with 3% fetal calf serum and 4 μM fura-2/AM. Cells were then washed and resuspended

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[1] The abbreviations used are: CF, cystic fibrosis; G protein, GTP binding protein; 2-CADO, 2-chloroadenosine; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; PMA, phorbol 12-myristate 13-acetate; CHA, N^6-cyclohexyladenosine; CPA, N^6-cyclopentyl adenosine; CGS-21680, 2-[4-(2-carboxyethyl)phenethylammino]-5′-N-ethylcarboxamido adenosine hydrochloride; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine.

in the same medium and left at room temperature until use. Before each experiment, an aliquot of cells was centrifuged and resuspended in Ca^{2+}-free saline solution containing 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 5.5 mM D-glucose, 20 mM Na-Hepes, pH 7.4, and 0.25 mM sulfinpirazone. Measurements of fluorescence were obtained with a Multiscan-2 spectrofluorimeter (AMKO-LTI) equipped with an alternating dual wavelength excitation system. The 350:385 ratio was converted to an actual $[Ca^{2+}]_i$ as described in Ref. 18, following the *in situ* calibration procedure reported in Ref. 19. Data are shown as representative experiments or as mean values ± S.E.

Electrophysiological Studies—Electrophysiological experiments were carried out with the "perforated-patch" modification of the patch clamp technique (20). With 56FHTE8o⁻, CFNPE9o⁻, and CFPEo⁻ cells, the series resistance after patch perforation was often higher than 20 MΩ. Under these conditions, the current clamp mode was used to determine the cell membrane potential. Continuous recordings were obtained with a chart recorder (Linseis 6512). Membrane resistance was monitored by pulsing the cells with −10 pA every 40 s. When possible, voltage clamp experiments were carried out. Membrane potential was held at −20 mV (Cl^- nernstian potential) and stepped every 5 s to −100 mV (K^+ nernstian potential) to monitor the activity of K^+ and Cl^- currents, respectively. Voltage clamp data were stored using either the chart recorder and a conventional VHS video cassette recorder, after digital conversion with a pulse code modulator (PCM-501ES, Sony). The standard extracellular solution contained 130 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, 10 mM Na-Hepes, pH 7.3. The pipette was filled with 50 mM KCl, 10 mM NaCl, 45 mM K_2SO_4, 1 mM $MgCl_2$, 0.5 mM EGTA, 10 mM Na-Hepes, pH 7.3, plus 200 μg/ml nystatin. Mannitol was added to the extracellular solution to maintain the osmolality at 300 mOsm/kg. Statistical analysis was performed with Student's *t* test.

Materials—ATP, 2-CADO, pertussis toxin, PMA, nystatin were purchased from Sigma; thapsigargin was from Calbiochem; CHA, CPA, CGS-21680, and DPCPX were from RBI, Natick, MA; and fura-2/AM was from Molecular Probes, Eugene, OR.

RESULTS

$[Ca^{2+}]_i$ Measurements in Human Airway Cells—The effect of purinergic compounds on $[Ca^{2+}]_i$ in CFNPE9o⁻ and CFPEo⁻ cells was similar to that previously described in 9HTEo⁻. Fig. 1*a* shows that in CFNPE9o⁻ cells, 100 μM ATP, administered in a Ca^{2+}-containing medium, caused an increase in $[Ca^{2+}]_i$ from the resting value of 60 ± 5 nM (n = 13) to 150 ± 11 nM (n = 4). Fig. 1*c* shows that the addition of 20 μM 2-CADO resulted in a $[Ca^{2+}]_i$ spike (255 ± 16 nM, n = 9), much larger and faster than that induced by ATP. The $[Ca^{2+}]_i$ spike was followed by a plateau which very slowly declined toward the resting value. A plateau was also caused by ATP (Fig. 1*a*), however it was less evident, due to the smaller relative increase in $[Ca^{2+}]_i$ caused by the nucleotide. These results were compared with those of parallel experiments carried out in Ca^{2+}-free, EGTA-containing medium. Under these conditions, the initial $[Ca^{2+}]_i$ spike caused by ATP and 2-CADO was largely maintained, indicating an intracellular origin, whereas the subsequent plateau was no longer evident (Fig. 1, *b* and *d*). This plateau would be expected for a process sustained by increased Ca^{2+} permeability at the plasma membrane.

Panels e and *f* of Fig. 1 illustrate the concentration dependence of the effect of 2-CADO and ATP, respectively, on $[Ca^{2+}]_i$ in CFNPE9o⁻ cells. Experiments were performed in Ca^{2+}-free, EGTA-containing medium. The maximal effect was observed at concentrations above 20–30 μM 2-CADO and 100 μM ATP. Similar results were also obtained with CFPEo⁻ and 9HTEo⁻ cells (data not shown).

The responses of the various cell lines to 100 μM ATP and 30 μM 2-CADO are compared to 9HTEo⁻ cells in Table I. The value of resting $[Ca^{2+}]_i$ was similar among the different cell lines. Conversely, variability in the extent of activation by 2-CADO was indicated, with CFNPE9o⁻ as the most active.

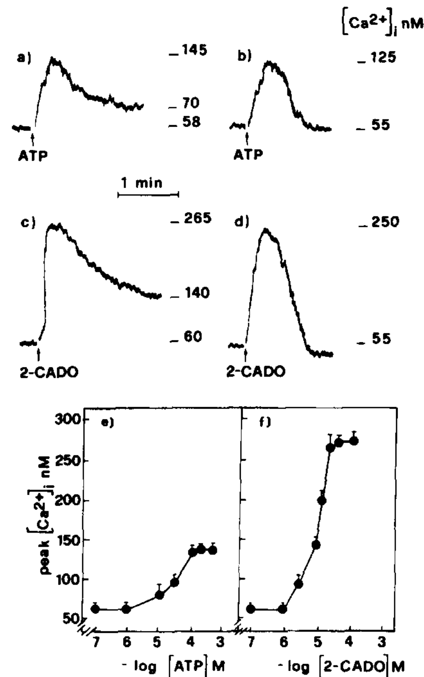

FIG. 1. **Effect of ATP and 2-CADO on $[Ca^{2+}]_i$.** Fura-2-loaded CFNPE9o⁻ cells (see "Experimental Procedures") were incubated in Ca^{2+}-containing saline solution (*a* and *c*) and in Ca^{2+}-free saline solution containing 0.1 mM EGTA (*b*, *d–f*). Where indicated, 100 μM ATP or 20 μM 2-CADO was added. In this and the following figures, $[Ca^{2+}]_i$ is reported on the *right*. Results shown are representative of four to nine highly consistent experiments. In *e* and *f*, data are presented as mean ± S.E. of the maximal (initial peak) values from four experiments.

TABLE I

Effect of 2-CADO and ATP on $[Ca^{2+}]_i$ of airway cell lines

Measurements were carried out in Ca^{2+}-free saline solution containing 0.1 mM EGTA.

Cell line	$[Ca^{2+}]_i$, nM		
	Control	+2-CADO	+ATP
		30 μM	*100 μM*
9HTEo⁻	60 ± 12	155 ± 18	95 ± 2
	(n = 44)	(n = 8)	(n = 4)
CFNPE9o⁻	55 ± 3	243 ± 16	128 ± 11
	(n = 30)	(n = 9)	(n = 7)
CFPEo⁻	52 ± 4	118 ± 3	73 ± 4
	(n = 21)	(n = 6)	(n = 6)

The effect of ATP was similar but weaker than that of 2-CADO in all the cell lines. In another normal cell line, 56FHTE8o⁻, a weak response to ATP or 2-CADO was detected (results not shown).

It has been reported that 2-CADO is a metabolically stable adenosine analog that binds to both adenosine receptors subtypes A_1 and A_2 (6). To assess which type of receptor is responsible for the response of 2-CADO, the effect on $[Ca^{2+}]_i$ of two A_1 adenosine agonists, CHA and CPA, was determined. At the concentration of 30 μM, both CPA and CHA caused an elevation of $[Ca^{2+}]_i$ due to release from intracellular stores (Fig. 2, *a* and *c*) and to increased plasma membrane Ca^{2+} permeability (Fig. 2, *b* and *d*). In all cell lines (CFNPE9o⁻, CFPEo⁻, and 9HTEo⁻), the rank order of nucleoside potency was 2-CADO > CHA ≥ CPA. The A_2 adenosine agonist, CGS-21680, was completely ineffective in elevating $[Ca^{2+}]_i$ (n = 3, Fig. 3*e*), however, CFNPE9o⁻ cells were sensitive to subsequent addition of 2-CADO. The CFPEo⁻ (n = 3) and 9HTEo⁻

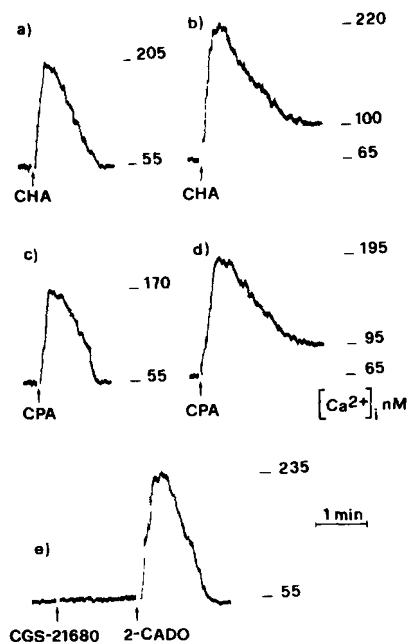

FIG. 2. **Effect of CHA, CPA, and CGS-21680 on $[Ca^{2+}]_i$.** Conditions as in Fig. 1; CFNPE9o⁻ cells were incubated in Ca^{2+}-free saline solution containing 0.1 mM EGTA (*a*, *c*, and *e*) and in Ca^{2+}-containing saline solution (*b* and *d*). CHA, CPA, CGS-21680, and 2-CADO were added at a concentration 30 μM. Results are representative of five highly consistent experiments.

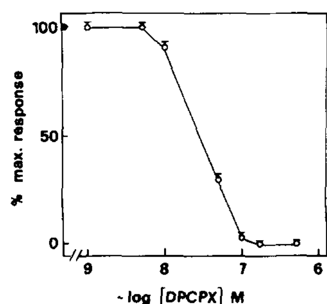

FIG. 3. **Dose-response of DPCPX inhibition of $[Ca^{2+}]_i$ elevation induced by 2-CADO.** Fura-2-loaded CFNPE9o⁻ cells were incubated in Ca^{2+}-containing saline solution. Data are expressed as percent of maximal $[Ca^{2+}]_i$ increase (mean ± S.E. of three experiments) caused by 30 μM 2-CADO.

cells ($n = 3$) showed no response to CGS-21680 (data not shown). Finally, 2-CADO-induced Ca^{2+} mobilization was completely blocked by the selective A_1 receptor antagonist DPCPX at concentrations higher than 10^{-7} M in CFNPE9o⁻ (Fig. 3) and in the other cell lines (not shown).

Other treatments were also used to characterize the $[Ca^{2+}]_i$ response in CFNPE9o⁻ cells. Preincubation with pertussis toxin (10 ng/ml, 22 h) resulted in a partial inhibition (30 ± 2%, $n = 3$) of the ATP-induced response, whereas the 2-CADO-induced response was almost completely inhibited (93 ± 3%, $n = 3$, data not shown). Pertussis toxin also inhibited the effect of CHA and CPA (97 ± 2 and 95 ± 3%, respectively, $n = 3$). Short term preincubation (1–3 min) with PMA (0.5 μM) completely inhibited the $[Ca^{2+}]_i$ transient release induced by either ATP and 2-CADO (data not shown). Pertussis toxin and PMA had similar effects in the other airway cell lines tested (not shown).

Membrane Potential Recordings of Human Airway Cells—Table II shows that 56FHTE8o⁻, CFNPE9o⁻, and CFPEo⁻ cells had a base-line membrane potential in the range of −40/−50 mV, while 9HTEo⁻ cells consistently showed more depolarized values.

After addition of 2-CADO and ATP, 9HTEo⁻ cells responded with a sustained depolarization (Fig. 4, *a* and *b*), consistent with the activation of Cl^- currents previously described (13). On the contrary, 56FHTE8o⁻, CFNPE9o⁻, and CFPEo⁻ responded to 2-CADO and ATP with a temporary (1–3 min) hyperpolarization, followed in many experiments by a slower depolarization. Representative responses are indicated in Fig. 4, *c* and *d*. Hyperpolarization was more marked in 56FHTE8o⁻ and CFNPE9o⁻ than in CFPEo⁻, while the depolarization was almost negligible in 56FHTE8o⁻ compared to CF cells (Table II).

The antagonist DPCPX (10^{-7} M) totally prevented the response of CFNPE9o⁻ cells to 20 μM 2-CADO ($n = 5$, results not shown). In control experiments performed in the same cell preparation, 2-CADO induced a hyperpolarization of −17 ± 3 mV ($n = 3$). DPCPX also antagonized the response to 2-CADO of CFPEo⁻ ($n = 6$) and 56FHTE8o⁻ ($n = 6$) cells (not shown). DPCPX alone had no effect on membrane potential (not shown).

Voltage Clamp Recordings of Human Airways Cells—Consistent with our previous findings (13), 2-CADO and ATP produced a sustained activation of Cl^- currents in 9HTEo⁻ cells, without any effect on K^+ currents (Fig. 5, *a* and *b*). This is apparent from the increase in downward deflections elicited by steps of membrane potential to −100 mV.

Contrary to the observations in 9HTEo⁻ cells, the other airway cell lines analyzed here showed a transient increase in current at −20 mV (Fig. 5, *c* and *d*), consistent with the activation of K^+ currents by 20 μM 2-CADO and 100 μM ATP. The time course of K^+ current activation coincides with that of the hyperpolarization detected in current clamp experiments (Fig. 4). In fact, the current at −20 mV began to increase a few seconds after application of the agonist, reached a maximum within 10–30 s and returned to resting values in 0.5–3 min.

2-CADO (Fig. 5*c*) and ATP (Fig. 5*d*) also activated the inward current detected at −100 mV, with a transient time course variable in duration from one cell to the other (1–5 min). The size of this current in 56FHTE8o⁻ and CF cells was significantly smaller ($p < 0.01$) than that observed in 9HTEo⁻ cells (Table III).

The identity of the inward current was investigated in experiments where the K^+ in the pipette solution was replaced by Na^+. As expected for a K^+ selective current, no increase of outward current at −20 mV was seen after application of 2-CADO or ATP (not shown). Under these conditions, the inward current was still detectable. In agreement with previous results obtained with 9HTEo⁻ cells (13), partial replacement of extracellular Cl^- with gluconate caused a shift in the reversal potential of membrane currents toward positive values, as expected for a Cl^--selective current (not shown). At high positive membrane potentials (*e.g.* +80 mV), the current showed a time-dependent decay (not shown). This is typical for Cl^- currents of the volume-sensitive type (13, 21).

Effect of Thapsigargin on $[Ca^{2+}]_i$ and Membrane Potential—The sesquiterpene lactone, thapsigargin, specifically blocks the endoplasmic reticulum Ca^{2+}-ATPase and is believed to mobilize Ca^{2+} ions by inhibiting Ca^{2+} sequestration by the inositol-1,4,5-trisphosphate (Ins-P_3)-sensitive stores (22, 23). Exposure of CFNPE9o⁻ cells to 1 μM thapsigargin in Ca^{2+}-free medium increased $[Ca^{2+}]_i$ from 55 ± 5 ($n = 6$) to 225 ± 20 nM ($n = 6$, Fig. 6). The effect of thapsigargin developed more slowly and persisted longer (7–8 min) than that induced by purinergic agonists. A 10-min pretreatment of the cells

TABLE II

Effect of 2-CADO and ATP on membrane potential recordings in airway epithelial cell lines

The values, given in mV (mean ± S.E.), are the peaks of hyperpolarization and depolarization, caused by 2-CADO and ATP with respect to the resting potential. The number of positive cells over the total number of experiments is indicated in parenthesis.

Cell line	Resting potential	2-CADO, 20 μM		ATP, 100 μM	
		Hyperpolarization	Depolarization	Hyperpolarization	Depolarization
9HTEo⁻	−18.9 ± 1.5 (12)	0 (0/5)	+9.0 ± 2.3 (5/5)	0 (0/3)	+6.7 ± 2.6 (3/3)
56FHTE8o⁻	−40.3 ± 1.5 (27)	−26.6 ± 3.0 (5/5)	+4.0 ± 0.5 (4/5)	−38.2 ± 3.7 (5/5)	0 (0/5)
CFNPE9o⁻	−43.3 ± 1.2 (58)	−27.9 ± 3.3 (7/7)	+18.0 ± 2.8 (7/7)	−24.9 ± 2.9 (9/12)	+11.2 ± 2.9 (9/12)
CFPEo⁻	−51.2 ± 1.2 (19)	−12.6 ± 3.3 (7/8)	+13.7 ± 3.0 (6/8)	−11.0 ± 3.0 (5/8)	+19.6 ± 3.8 (8/8)

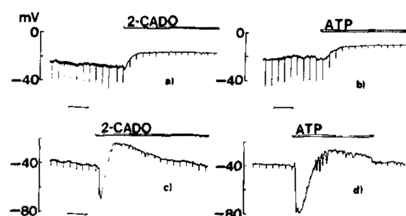

FIG. 4. **Effect of 2-CADO and ATP on airway epithelial cell membrane potential.** The *traces* represent the time course of membrane potential upon addition of 20 μM 2-CADO or 100 μM ATP. The downward deflections of traces are the result of periodic current pulses, as described under "Experimental Procedures." The *horizontal scale bars* show an interval of 2 min. *Panels a* and *b* are from two 9HTEo⁻ cells; *panels c* and *d* from two CFNPE9o⁻ cells.

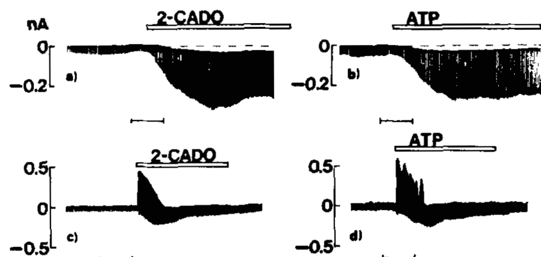

FIG. 5. **Effect of 2-CADO and ATP on membrane currents in airway epithelial cells.** The time course of membrane currents elicited at −20 and −100 mV. K⁺ and Cl⁻ currents, respectively, were simultaneously monitored. Where indicated, 20 μM 2-CADO or 100 μM ATP was added. *Traces a* and *b* were from 9HTEo⁻ cells; *traces c* and *d* from CFNPE9o⁻ cells. The *horizontal scale bars* show an interval of 2 min.

TABLE III

Effect of 2-CADO and ATP on perforated patch whole cell K⁺ and Cl⁻ currents in airway epithelial cell lines

$\Delta I_{(-20mV)}$ and $\Delta I_{(-100mV)}$ are the increases of membrane currents detected at −20 and −100 mV, respectively (see "Experimental Procedures"). The values are given as mean ± S.E. The number of positive cells over the total number of experiments is indicated in parenthesis.

Cell line	2-CADO, 20 μM		ATP, 100 μM	
	$\Delta I_{(-20mV)}$	$\Delta I_{(-100mV)}$	$\Delta I_{(-20mV)}$	$\Delta I_{(-100mV)}$
	pA			
9HTEo⁻	0 (0/15)	−345 ± 33 (15/15)	0 (0/6)	−375 ± 27 (6/6)
56FHTE8o⁻	+390 ± 112 (4/6)	−80 ± 20 (3/6)	+447 ± 38 (3/5)	−40 ± 6 (3/5)
CFNPE9o⁻	+229 ± 29 (17/25)	−111 ± 19 (16/25)	+290 ± 34 (8/18)	−73 ± 20 (12/18)
CFPEo⁻	+120 ± 34 (4/6)	−208 ± 27 (5/6)	+149 ± 47 (4/4)	−140 ± 20 (4/4)

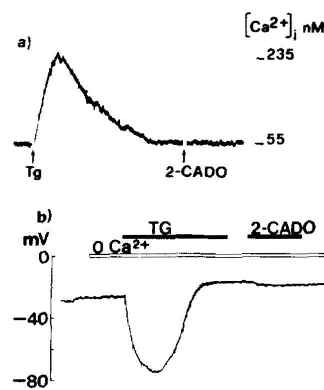

FIG. 6. **Effect of thapsigargin on $[Ca^{2+}]_i$ (*a*) and membrane potential (*b*) changes induced by 2-CADO.** 1 μM thapsigargin (*Tg*) was administered to CFNPE9o⁻ cells, followed by 30 μM 2-CADO. The *horizontal scale bar* shows an interval of 2 min. Results shown are representative of four (*a*) and 13 (*b*) highly consistent experiments.

with 1 μM thapsigargin resulted in abolition of the 2-CADO-induced (Fig. 6*a*) and ATP-induced (not shown) $[Ca^{2+}]_i$ responses. The effect of thapsigargin was similar in all the cell lines here analyzed (not shown).

Thapsigargin (1 μM) also caused a transient hyperpolarization in perforated-patch experiments (Fig. 6*b*). The addition of 20 μM 2-CADO 8–10 min after thapsigargin application was completely inefficient in 56FHTE8o⁻ (n = 9, Fig. 6*b*), CFPEo⁻ (n = 5) and CFNPE9o⁻ (n = 13). The effect of ATP was also abolished by thapsigargin treatment (not shown) in CFNPE9o⁻ (n = 5), CFPEo⁻ (n = 5), and 56FHTE8o⁻ (n = 5).

DISCUSSION

The present study confirms and greatly extends our previous work (13) showing that cell lines derived from human airway epithelium respond to both P_1 and P_2 purinergic agonists with an increase in $[Ca^{2+}]_i$ and activation of membrane currents. Moreover, the work described here expands previous studies through the use of adenosine analogs (4, 5).

2-CADO, which binds to both A_1 and A_2 subtypes of adenosine receptors (6), was the most effective nucleoside for inducing $[Ca^{2+}]_i$ elevation. The effect of 2-CADO was blocked by low concentration of the selective adenosine A_1 antagonist DPCPX, clearly indicating that the effect is mediated via typical adenosine A_1 receptors. Furthermore, selective A_1 adenosine agonists CHA and CPA mobilized intracellular Ca^{2+}, whereas an A_2 agonist (CGS-21680) was completely

inefficient. The ATP-induced $[Ca^{2+}]_i$ increase was lower than that elicited by 2-CADO.

Although Ins-P_3 formation was not directly determined, our data suggest that binding to both P_1 and P_2 receptors increases $[Ca^{2+}]_i$ through a transduction mechanism that involves G proteins and probably phospholipase C. This hypothesis is substantiated by the following findings: 1) the effect of ATP and 2-CADO was completely inhibited by pretreatment with thapsigargin; 2) the effect of ATP or 2-CADO was completely inhibited by activation of protein kinase C; and 3) the effect of ATP was partially inhibited and the effect of 2-CADO was completely blocked by pertussis toxin.

The finding that thapsigargin completely abolished the response to both ATP and 2-CADO supports the notion that these two compounds released Ca^{2+} from the same intracellular compartment that was depleted by thapsigargin, *i.e.* the Ins-P_3-sensitive store (22).

Short term activation of protein kinase C by PMA also abolished the response of both ATP and 2-CADO. This feature is typical of many receptor-mediated phospholipase C signal-transduction systems (24). The sensitivity to pertussis toxin of adenosine receptor- and ATP receptor-mediated increases in $[Ca^{2+}]_i$ indicates G protein involvement. In smooth muscle cells (10) and rat mast cells (9), it has been reported that A_1 adenosine receptors are coupled to phospholipase C through a mechanism completely blocked by pertussis toxin. Conversely, in the same cells, ATP response was only partially sensitive to treatment with pertussis toxin (10). These findings have been considered as evidence for the presence of a receptor coupled to two types of G proteins, one being sensitive to pertussis toxin treatment, the other not (10).

In addition to fura-2 experiments, we have carried out perforated patch recordings to ascertain the effect of 2-CADO and ATP on the membrane conductance of CFNPE9o$^-$, CFPEo$^-$, and 56FHTE8o$^-$ cells. In a previous study, we showed that both compounds elicited a sustained activation of volume-sensitive Cl^- currents in 9HTEo$^-$ cells. Surprisingly, the other airway cell lines examined here showed a different behavior. 56FHTE8o$^-$, CFNPE9o$^-$ and, to a lesser extent, CFPEo$^-$ responded to either 2-CADO and ATP with a transient activation of K^+ currents, as revealed by current clamp and voltage clamp recordings. The effect of 2-CADO was due to binding to A_1 adenosine receptor, since it was abolished by DPCPX. The observation that the changes in membrane potential caused by 2-CADO and ATP were abolished by preincubation with thapsigargin suggests that the response of the two compounds is mediated by the increase in $[Ca^{2+}]_i$. Nevertheless, an apparent discrepancy between Ca^{2+} measurements and electrophysiological recordings consists in the finding that ATP was less effective than 2-CADO as a Ca^{2+} elevating agent, whereas both compounds were equally effective in modifying K^+ currents. If K^+ currents were Ca^{2+}-dependent, the modest elevation of Ca^{2+} caused by ATP might be sufficient for activation of K^+ currents. This could result from either a localized Ca^{2+} spike, or from a low activation threshold of K^+ channels for Ca^{2+} ions. In lacrimal acinar cells, it has been reported that a $[Ca^{2+}]_i$ change of twice the resting value, restricted to the luminal membrane, was able to fully activate the K^+ channels (25). In addition, activation of K^+ channels by adenosine has been reported to be directly mediated through G proteins in heart muscle cells (26). If K^+ channel activation were Ca^{2+}-independent, it would explain the result with the 56FHTE8o$^-$ cell line, in which ATP and 2-CADO failed to significantly increase $[Ca^{2+}]_i$, but were effective in activating K^+ currents. However, it cannot yet be ruled out that K^+ currents in the cells studied are not Ca^{2+} dependent, but are rather regulated by other transduction mechanisms.

The stimulation of K^+ currents by extracellular purinergic agonists such as ATP and adenosine A_1 receptor agonists is a new result for the airway epithelium. Such an effect is important, since K^+ exit through basolateral channels could provide the driving force for Cl^- secretion through the apical membrane.

Three types of Cl^- currents can be distinguished in human epithelial cells on the basis of the shape of their current voltage relationship and kinetics. These currents are separately regulated by cAMP, Ca^{2+}, and cell volume (27). The Cl^- current activated by 2-CADO and ATP in the cell lines examined in this study belongs to the volume-sensitive type. In this respect, it has been recently reported that in a rabbit cortical collecting duct cell line, stimulation of adenosine A_1 receptors activates a volume-sensitive Cl^- channel, through a complex pathway involving phospholipase C, protein kinase C, and a G protein (28). These authors have postulated that during ischemia, autocrine and paracrine release of adenosine from renal cells activates Cl^- channels. Opening of Cl^- channels would then restore cell volume that is increased during ischemia. It would be interesting to test this hypothesis also in airway epithelium.

It is not yet possible to explain the peculiar behavior of 9HTEo$^-$ cells with respect to the other cell lines examined here. It is possible that the differences are due to clonal variation or cell-type origin.

In a recent paper, Lazarowski *et al.* (29) have found that adenosine analogs stimulate intracellular cAMP accumulation and increase short circuit current in human airway epithelial cells. The presence of an A_2 receptor positively coupled to adenylate cyclase was postulated (29).

In addition to the classical mechanism involving adenylate cyclase, our data indicate clearly that adenosine might act in airway epithelium through another transduction mechanism, which causes $[Ca^{2+}]_i$ increase and activation of K^+ and volume-sensitive Cl^- channels. Moreover, this transduction mechanism appears to be intact in CF airway cells. Although extrapulmonary manifestations of CF must not be neglected, 95% of the morbidity and mortality of the disease arises from the pulmonary complications, and most approaches to therapy are focused on these (1). Accordingly, identification of agonists able to bypass the defective cAMP- dependent regulation of the Cl^- channel might represent an useful tool for development of new pharmacological approaches.

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