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Regulation of transepithelial ion transport and intracellular calcium by extracellular ATP in human normal and cystic fibrosis airway epithelium

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1 The role of extracellular nucleotides in regulation of ion transport activities (short circuit current, I_{sc}) of human respiratory epithelia was studied.

2 Application of nucleotides to the apical or basolateral membrane of human nasal epithelium induced a concentration-dependent increase in I_{sc}.

3 The rank order of potency of purine- or pyrimidine-induced changes in I_{sc} of normal human nasal epithelium when applied to the apical membrane (UTP $\geqslant$ ATP > ATPγS > 2MeSATP > ADPβS $\gg$ $\beta\gamma$MeATP $\geqslant$ $\alpha\beta$MeATP) or basolateral membrane (2MeSATP > UTP > ATP > ATPγS > $\alpha\beta$MeATP > $\beta\gamma$MeATP) is consistent with involvement of a P_2 purinoceptor. A similar rank order of potencies was observed for nucleotide effects on intracellular calcium measured by Fura-2 fluorescence using microspectrofluorimetry.

4 Similar nucleotide potency in the regulation of ion transport and intracellular calcium in cystic fibrosis (CF) airway epithelium (UTP $\geqslant$ ATP) was observed, suggesting purinoceptors might be used to stimulate ion transport processes that would promote hydration of airway secretions and facilitate their clearance from CF lungs.

5 These data provide evidence for the regulation of ion transport by P_2 purinoceptors in normal and cystic fibrosis human airway epithelium.

Keywords: Epithelium; adenosine triphosphate; purinoceptors; airway epithelium; cystic fibrosis; ion transport; purine nucleotide; pyrimidine nucleotide; calcium release

Introduction

Extracellular adenosine triphosphate (ATP) has been shown to regulate a variety of biological processes including non-vascular smooth muscle contraction (Maguire & Satchell, 1979; Brown & Burnstock, 1981) and vascular tone (Burnstock & Kennedy, 1986; Haeussinger *et al.*, 1987), platelet aggregation (Born & Kratzer, 1984), neurotransmission (Burnstock, 1971; Burnstock & Sneddon, 1985), and cellular ion transport (Burgess *et al.*, 1979; Gallacher, 1982) and secretory activities (Chapal & Loubatières-Mariani, 1981; Pearson *et al.*, 1983). These effects are mediated by specific purinoceptors which respond to ATP or other nucleotides present in the extracellular millieu (Gordon, 1986).

Purinoceptors have been functionally identified in rat pulmonary epithelia in studies of regulation of alveolar Type II surfactant phospholipid secretion (Rice & Singleton, 1986). To our knowledge these receptors have not been described in human airway epithelial cells. Because ion transport appears to be regulated by purinoceptor stimulation in other eipthelia (Burgess *et al.*, 1979; Gallacher, 1982), we investigated several features of the effect of extracellular nucleotides on the ion transport activities of human airway epithelium.

Purinoceptor regulation of ion transport might have potential therapeutic benefit in lung diseases characterized by abnormalities in epithelial ion transport, e.g., cystic fibrosis. In cystic fibrosis (CF) the airway epithelial dysfunction is expressed in part by defective regulation of Cl^- ion transport by secretagogues that regulate the apical cell membrane Cl^- channel by adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP)-dependent or protein kinase C-dependent mechanisms (Boucher *et al.*, 1986, 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989). Induction of Cl^- secretion by CF airway epithelia *in vivo* would be expected to help liquify the relatively dehydrated, thick airway surface liquid that characterizes this disease. We therefore tested whether nucleotides would bypass regulatory defects in CF airway epithelia and induce Cl^- secretion at rates similar to those of normal airway cells. Consequently, this study could suggest a new class of therapeutic agents for the treatment of CF.

Methods

Cell culture

Freshly excised human nasal epithelium from normal or cystic fibrosis subjects was grown in primary culture in F-12, hormone-supplemented medium as previously described (Wu *et al.*, 1985). For $[Ca^{2+}]_i$ measurements, cultures were grown on non-fluorescent vitrogen substrates on glass coverslips. Cultures for electrophysiological studies were grown to confluence on permeable collagen matrix supports (CMS), allowing addition of agonists to the basolateral or apical surface of the epithelial sheet.

Biolectric studies

Confluent monolayers of primary human nasal epithelium were mounted in modified Ussing chambers (Knowles *et al.*, 1983; Boucher *et al.*, 1988). The studies were performed under short circuit current conditions at 37°C, and in some studies, amiloride was added to the apical bath (10^{-4} M) to block transepithelial sodium transport. Changes in transepithelial potential difference (V_t), resistance (R_t) and short circuit current (I_{sc}) were measured in response to addition of various nucleotides. Each cultured preparation was exposed to only one concentration of an agonist on either the basolateral or apical surface to avoid the tachyphylaxis observed in preliminary cumulative dose-response studies. To construct concentration-effect relationships of responses to nucleotides, we assumed that the same maximum response to an agonist could be induced from each tissue culture preparation from the same individual (see Statistics).

Measurements of intracellular calcium

Primary human nasal epithelial cells grown to confluence on vitrogen coated coverslips were loaded with a final concentration of 3 μM Fura-2/AM at 37°C for 30 min. The cells were then washed in NaCl Ringer and mounted in a chamber for measurements of fluorescence. To reduce the rate of leakage of Fura-2 from the cell into the extracellular space and avoid time-dependent compartmentalization of the probe, all measurements of $[Ca^{2+}]_i$ were performed at 25°C. At this temperature, no vesicular bright spots indicative of compartmentalization of the probe were observed.

Measurements of $[Ca^{2+}]_i$ in single human nasal epithelial cells were obtained with a modular microspectrofluorimeter (SPEX Industries, Inc., Edison, NJ, U.S.A.) attached to a Zeiss Axiovert IM 35 microscope. The system was equipped with a xenon lamp, beam splitter, two monochromators and a rotating chopper mirror that permitted excitation of cell fluorescence at alternating wavelengths of 340 and 380 nm (emission $\geqslant$ 450 nm). The fluorescent signal from a single cell was measured with a photometer equipped with a pinhole (spot diameter of 3–5 μm) that excluded signals from adjacent cells.

After agonist was added, the fluorescent signal was quenched by a NaCl Ringer solution containing 1.5×10^{-4} M digitonin and 10^{-3} M $MnCl_2$. The remaining signal at each excitation wavelength, equivalent to the background fluorescence in non-loaded cells, was subtracted from data from Fura-2/AM loaded cells before the ratio (340 nm/380 nm) was taken. The 340 nm/380 nm ratio was converted to an actual $[Ca^{2+}]_i$ measurement by using the external calibration standards and the formula derived by Grynkiewicz et al. (1985) used with dual wavelength measurements: $[Ca^{2+}]_i = K[(R_x - R_o)/(R_s - R_x)]$, with R_o and R_s representing the ratios at 0 Ca^{2+} and saturating Ca^{2+}, respectively. R_x represents the experimental ratio. K is $K_d(F_o/F_s)$, with $K_d = 1.57 \times 10^{-7}$ M at 25°C as the effective dissociation constant for Fura-2, and F_o and F_s represent the fluorescence intensities at 380 nm with 0 and saturating Ca^{2+}, respectively.

Statistical methods

A relatively large variability in the maximal responses was observed in tissues from different donors. However, the responses (I_{sc} or $[Ca^{2+}]_i$) within preparations from a single donor were more homogeneous. To control for inter-donor tissue variability, tissues from a single donor were used for the entire concentration-range of a nucleotide agonist. Only tissues with transepithelial potential difference greater than -5 mV and transepithelial resistance greater than 100 ohms cm^{-2} were used in the studies. For comparing effectiveness of ATP administered to the luminal versus basolateral surface of normal and CF preparations, the absolute changes in I_{sc} from basal levels are plotted. For comparison of rank orders of potency for nucleotides, the percentage change in I_{sc} from basal levels is shown. For measurements of $[Ca^{2+}]_i$, only absolute changes from basal levels are plotted. To estimate a representative variance for the response to agonists, we (1), determined the absolute response to the maximum agonist concentration; (2) calculated the percentage of the maximal response yielded by each lower agonist concentration (normalized maximum percentage response); and (3) calculated the variance of the normalized responses for each agonist concentration in preparations from different donors. Rank orders of potency of the agonists were obtained by comparing EC_{50}s of each agonist.

Chemicals and solutions

ATP, UTP, ATPγS, CTP, GTP, ITP, adenylyl-imidodiphosphate (AMPPNP), β,γ-methylene ATP (β,γ-MeATP), ADPβS, ADP, AMP, UDP, 5-BrUTP, UMP, ATPαS and dipyridamole were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN, U.S.A.). α,β-Methylene ATP (α,β-MeATP) and amiloride were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). 2-Methylthio ATP (2MeSATP) was purchased from Research Biochemicals Inc. (Natick, MA, U.S.A.).

Bioelectric properties were measured with confluent monolayers bathed by Ham's F-12 culture solution without hormone supplements (Gibco, Grand Island, NY, U.S.A.).

The acetoxymethylester of Fura-2 (Fura-2/AM) and the pentapotassium salt Fura-2 were purchased from Molecular Probes (Eugene, OR, U.S.A.). To obtain external calibration standards for the dye, the pentapotassium salt Fura-2 was used at 15 μM in solution with 150 mM KCl, 20 mM NaCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), and either 5 mM $CaCl_2$ or 2 mM EGTA.

For intracellular calcium studies, NaCl Ringer solution at 25°C was employed, containing the following (in mM): NaCl 150, KCl 5, D-glucose 5, HEPES 10, $CaCl_2$ 2, $MgCl_2$ 2, adjusted to pH 7.4. For studies to determine the ionic form of ATP active at the receptor, $MgCl_2$ was varied in the above NaCl Ringer between nominally Mg^{2+}-free, 2 mM and 5 mM Mg^{2+}. For studies with solutions free of calcium, 2 mM EGTA replaced the $CaCl_2$.

Results

Normal human nasal epithelium-ion transport

The effects of extracellular ATP on normal human nasal epithelium were investigated employing biolectric measurements of ion transport activity when the nucleotide was applied to either the apical or basolateral membrane. Representative tracings obtained after application of ATP to cultured sheets of human nasal epithelium mounted in Ussing chambers are illustrated in Figure 1. Figure 1(a) and (b) represents the I_{sc} response when ATP is applied to the apical or basolateral membrane of tissues in the basal, Na^+ absorptive mode (Boucher et al., 1986). ATP rapidly stimulated an increase in I_{sc} when applied to either surface. In general, the change in I_{sc} induced by application of ATP to the apical or the basolateral side returned to baseline or below within 5 min after addition of agonist. Oscillations in I_{sc} following ATP were frequently observed. Apical pretreatment with amiloride (10^{-4} M) removes active Na^+ absorption as a component of the I_{sc} so that the residual I_{sc} reflects a Cl^- secretory current (Boucher et al., 1986; Willumsen et al., 1989). Figure 1(c) and (d) represent the typical Cl^- secretory response of human nasal epithelium when ATP is applied to amiloride-pretreated tissues. Following the initial peak, the ATP induced increase in I_{sc} after basolateral addition to amiloride-pretreated tissues returned to baseline within 5 min (Figure 1d). In contrast, most tissues (91% of those followed for 10 min) treated with ATP on the apical surface following amiloride pretreatment exhibited prolonged (> 10 min) increases in I_{sc} above baseline levels (Figure 1c).

The concentration-effect relationship of ATP applied to normal human nasal epithelium under basal conditions is shown in Figure 2. Comparisons were made between responses of tissues from different donors based on the peak change in I_{sc} following ATP application (see Figure 1). The curve describes the mean peak change in I_{sc} in response to increasing log concentrations of ATP applied to the apical or basolateral surface. The effectiveness of the nucleotide is approximately equal when applied to the apical or basolateral surface between 10^{-7} and 10^{-4} M. A large increase in I_{sc} is seen with 10^{-3} M ATP applied to the basolateral membrane that is not seen with apical application.

Figure 3 illustrates concentration-effect relationships for ATP applied to the apical or basolateral membrane of amiloride-pretreated tissues. Again, the effect of ATP on ion transport is shown as the mean peak change in I_{sc} after ATP application. The change in ion transport induced by ATP in

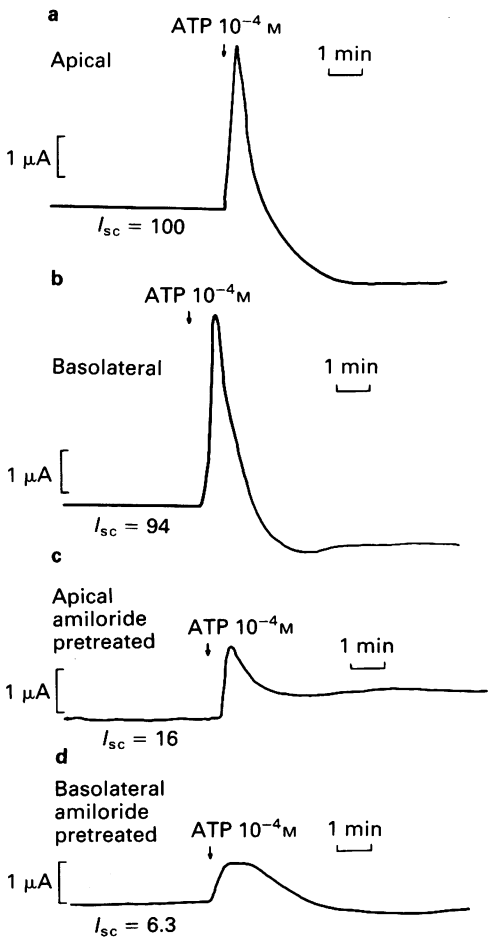

Figure 1 Representative tracings of effect on I_{sc} of extracellular ATP (10^{-4} M) applied to the apical or basolateral membrane of human nasal epithelium. (a) Response of human nasal epithelium in basal transport mode (primarily Na^+ absorptive) to ATP applied to the apical surface (basal I_{sc} = 100 μA cm^{-2}). (b) Response of human nasal epithelium in basal mode to ATP applied to the basolateral surface (basal I_{sc} = 94 μA cm^{-2}). (c) Cl^- secretory response to ATP applied to apical surface of amiloride-pretreated tissue (post-amiloride I_{sc} = 16 μA cm^{-2}). (d) Cl^- secretory response to ATP applied to the basolateral surface of amiloride-pretreated tissue (post-amiloride I_{sc} = 6 μA cm^{-2}).

amiloride-pretreated tissues is routinely smaller than that observed in tissues in the basal state (Figure 2). The potency and effectiveness of ATP in amiloride-treated tissues are similar whether applied to the apical or basolateral membrane and the log concentration-effect curves are sigmoidal in character, with EC_{50} values of approximately 1–2 × 10^{-5} M.

The purinoceptor subtype(s) linked to regulation of ion transport of human nasal epithelium were characterized by obtaining concentration-effect relationships for a variety of purine and pyrimidine agonists in preparations derived from normal and CF patients. We characterized receptor subtype(s) on the basolateral surface by measuring nucleotide effect on basal Na^+ transport rates. Because therapies designed to induce Cl^- secretion might best be delivered by the aerosol route, receptor subtype characterization on the apical barrier was performed in the presence of amiloride. The effect of extracellular nucleotides on ion transport is shown as the percentage change in I_{sc} from control values when applied to the basolateral or apical surface of the culture. Basal pre-agonist currents were similar for tissues in each concentration group (see Figure legends).

Under the culture conditions employed in these studies, the P_1 receptor agonist adenosine, following preincubation of tissues with dipyridamole (10^{-6} M) to block adenosine uptake, induced only small and variable changes in I_{sc} compared to

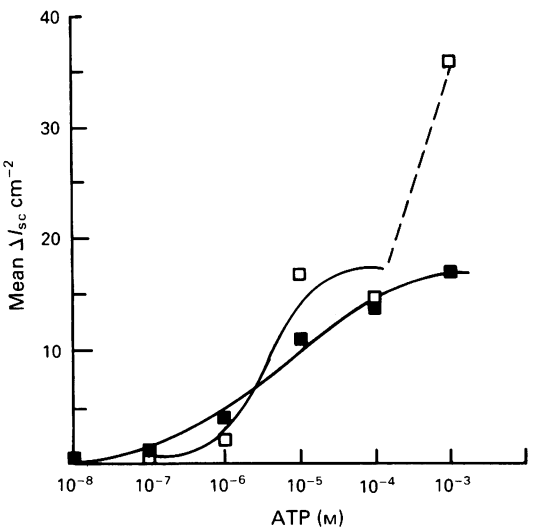

Figure 2 Log concentration-effect relationship (mean change in I_{sc} from basal levels) of ATP applied to the apical (■) or basolateral (□) surface of human nasal epithelium without amiloride-pretreatment. Mean I_{sc} of tissues in basal mode for range of ATP concentrations tested were (in μA cm^{-2}): apical ATP 80 ± 2, range 16–150, n = 3–9 at each agonist concentration; basolateral ATP 68 ± 5, range 17–138, n = 3–13 at each agonist concentration. S.e.mean of each data point is ≤12% of the normalized maximum response.

ATP (compare with Figures 4 and 5, below). Addition of adenosine to the apical surface of amiloride-pretreated human nasal epithelium at 10^{-5} M (n = 7) or 10^{-4} M (n = 8) induced an increase of 10 ± 7 or 10 ± 6%, respectively, whereas addition of the same doses to the basolateral barrier (n = 8, each dose) raised I_{sc} by less than 5%. These findings suggest that the activation of P_1 receptors contributes little to the measured effects of extracellularly applied ATP on ion transport. Therefore, we focused on agonists that interact with P_2 receptors in the regulation of ion transport and $[Ca^{2+}]_i$ mobilization in human nasal epithelium.

Figure 4 illustrates the concentration-effect relationships of agonists applied to the basolateral surface of airway epithe-

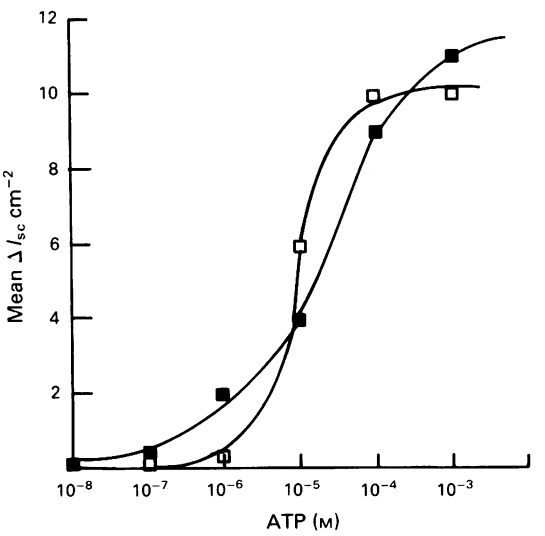

Figure 3 Log concentration-effect relationships (mean change in I_{sc} over basal levels) of ATP applied to the apical (■) or basolateral (□) membrane of amiloride-pretreated (10^{-4} M) human nasal epithelium. Mean I_{sc} of amiloride-pretreated tissues for the range of ATP concentrations tested were (in μA cm^{-2}): apical ATP, 13 ± 1, range 9–16, n = 3–11 at each agonist concentration; basolateral ATP, 14 ± 1, range 8–28, n = 3–6 at each agonist concentration. S.e.mean of each data point is ≤12% of the normalized maximum response.

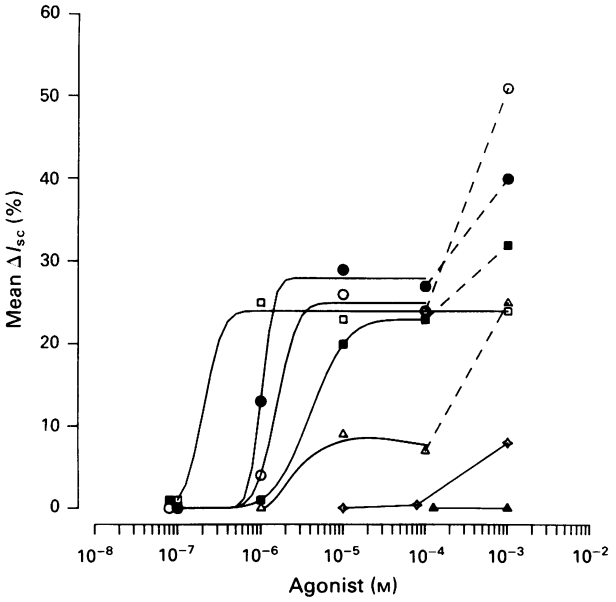

Figure 4 Log concentration-effect curves (percentage change in I_{sc} from basal levels) of nucleotides applied to the basolateral surface of normal human nasal epithelium. Mean basal I_{sc} in each set of experiments were (in $\mu A\,cm^{-2}$): ATP (○), 69 ± 5, range 55–80, $n = 5$ ($n = 3$–11 at each agonist concentration); UTP (●), 66 ± 9, range 21–158, $n = 9$ ($n = 3$–23); 2MeSATP (□), 48 ± 6, range 35–65, $n = 6$ ($n = 3$–17); ATPγS (■), 47 ± 8, range 28–72, $n = 3$ ($n = 3$); ADPβS (△), 49 ± 6, range 36–65, $n = 3$ ($n = 3$); αβMeATP (◇), 51 ± 12, range 28–66, $n = 3$ ($n = 3$); βγMeATP (▲), 79 ± 21, range 59–100, $n = 3$ ($n = 3$). S.e.mean of each data point is ≤15% of the normalized maximum response.

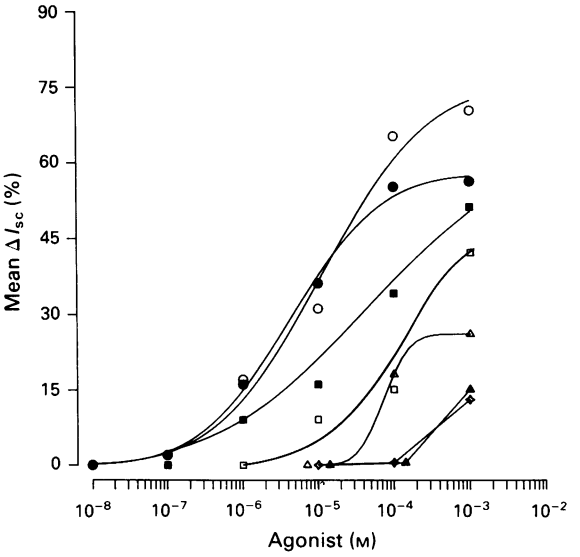

Figure 5 Log concentration-effect curves (percentage change in I_{sc} from basal levels) of nucleotides applied to the apical surface of human nasal epithelium pretreated with amiloride (10^{-4} M). Mean post-amiloride I_{sc} in each set of experiments were (in $\mu A\,cm^{-2}$): ATP (○), 13 ± 1, range 9–16, $n = 8$ ($n = 3$–11 at each agonist concentration); UTP (●), 12 ± 1, range 11–15, $n = 5$ ($n = 3$–17); ATPγS (■), 12 ± 1, range 8–15, $n = 3$ ($n = 3$); 2MeSATP (□), 16 ± 2, range 12–21, $n = 3$; ($n = 3$–7); ADPβS (△), 15 ± 1, range 13–16, $n = 3$ ($n = 3$) βγMeATP (▲), 14 ± 0, range 8–19, $n = 3$ ($n = 3$); αβMeATP (◇), 11 ± 0, range 7–14, $n = 3$ ($n = 3$). S.e.mean of each data point is ≤13% of the normalized maximum response.

lium. Compared to the concentration-effect curve of ATP, agonists that stimulate P_{2x} receptors (αβMeATP and βγMeATP) induced little change in ion transport rates. The observed rank order of potency for agonists that significantly increased I_{sc} was 2MeSATP > UTP ⩾ ATP > ATPγs > ADP > ADPβS. At concentrations of ATP, UTP, ATPγS or ADPβS between 10^{-7} and 10^{-4} M, the relationship between log agonist concentration and observed responses was a curve of sigmoidal character. The concentration-effect curve of these agonists was biphasic in character when the effect on I_{sc} of 10^{-3} M drug was considered.

The concentration-effect relationships for nucleotides added to the apical surface of amiloride-pretreated tissues are shown in Figure 5. Compounds reported to be effective P_{2x} receptor agonists produced little change in ion transport by airway epithelium. Those reported to be effective P_{2y} or UTP-sensitive receptor agonists stimulated Cl^- secretion with the following rank order of potency: ATP ⩾ UTP > ATPγS > ADP > 2MeSATP > ADPβS.

In an attempt to test whether multiple P_2 receptor types are expressed on each membrane, or a single novel receptor subtype is expressed on either barrier, additivity studies were performed using two ligands that supposedly interact with separate receptor subtypes, UTP and 2MeSATP. Co-addition of saturating concentrations of 2MeSATP (10^{-4} M) and UTP (10^{-4} M) on the basolateral membrane induced an effect that was about twice that induced by each of the agonists alone in sets of tissue preparations obtained from three separate donors. The mean increase in I_{sc} for UTP alone was 4.6 ± 0.5 $\mu A\,cm^{-2}$, and for 2MeSATP alone was 4.3 ± 0.9 $\mu A\,cm^{-2}$, whereas current increased 10 ± 1 $\mu A\,cm^{-2}$ in response to the combination of UTP and 2MeSATP ($n = 3$). The additivity observed suggests each ligand interacts with a separate receptor on the basolateral membrane. When the two agonists were applied to the apical surface singly and in combination following amiloride, no additive effects were evident.

Normal human nasal epithelium-intracellular calcium

Based on studies in other epithelia indicating that regulation of $[Ca^{2+}]_i$ by purinoceptors initiates changes in ion transport rates (Kimmich & Randles, 1982), we investigated whether ATP regulated $[Ca^{2+}]_i$ in single human nasal epithelial cells using an intracellular Ca^{2+}-sensitive fluorescent dye. Extracellular application of ATP induced an immediate increase in $[Ca^{2+}]_i$ levels that decreased over 1 to 2 min to a prolonged plateau (Figure 6a). Exposure to ATP in Ca^{2+}-free medium resulted in an initial sharp increase in $[Ca^{2+}]_i$ which returned to baseline over a 2 min period with no plateau phase observed (Figure 6b). Return of the cells to a Ca^{2+}-containing solution resulted in restoration of the plateau phase in the Ca^{2+} response to ATP.

To investigate whether changes in $[Ca^{2+}]_i$ might be related to regulation of ion transport, receptor characterization was performed by measuring changes in $[Ca^{2+}]_i$ in response to a number of nucleotide drugs. Concentration-effect curves for angonists active at P_{2x}, P_{2y} subtype or UTP-sensitive receptors and other purine or pyrimidine receptor agonists were generated, measuring mean change in $[Ca^{2+}]_i$ in response to agonist concentration (Figure 7). ATP, UTP and ATPγS were the most effective agonists. Classical P_{2x} (αβMeATP and βγMeATP) and P_{2y} (2MeSATP and ADPβS) receptor agonists had little effect (Figure 7a) as did other analogues of ATP and ADP (Figure 7b). 5BrUTP was essentially as effective as UTP for stimulation of Ca^{2+} mobilization (Figure 7c).

To characterize the chemical form of ATP interacting with the purinoceptor on human nasal epithelium, fluorescence measurements of single cell $[Ca^{2+}]_i$ response to ATP (10^{-3} to 10^{-8} M) were obtained with various levels of Mg^{2+} in the extracellular medium. Increasing $[Mg^{2+}]$ resulted in a shift to the right of the dose-response curve: the EC_{50} values for ATP were 5×10^{-6} M in solutions nominally Mg^{2+}-free; 2×10^{-5} M in solutions containing 2 mM Mg^{2+}; and 1×10^{-4} M in solutions containing 5 mM Mg^{2+} ($n = 4$ for each Mg^{2+} concentration). These shifts suggest that the free acid form ATP^{4-} is the active form at human airway P_2 receptors

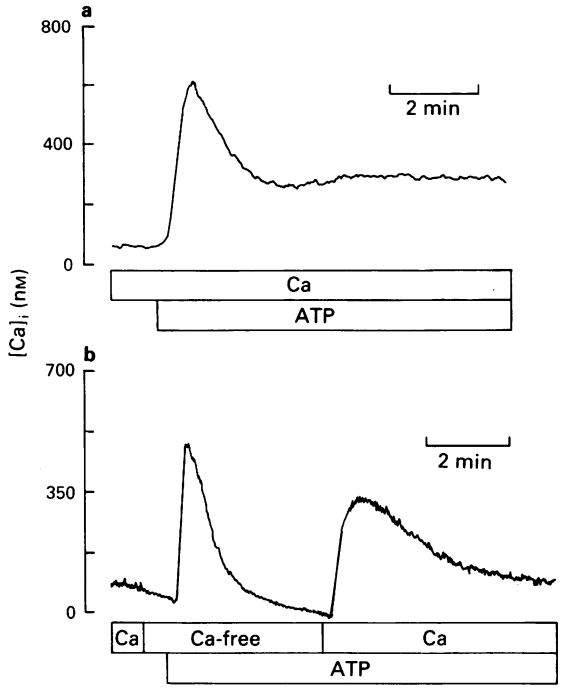

Figure 6 The effect of ATP (10^{-4} M) on the concentration of intracellular calcium $[Ca^{2+}]_i$ in single human nasal epithelial cells with (a) and without (b) 2 mM Ca^{2+} in the bathing medium. In (b) external Ca^{2+} was restored to the culture medium after the peak response to ATP, resulting in a plateau level above baseline. The increase in $[Ca^{2+}]_i$ is calculated from the change in fluorescence due to increased binding of free Ca^{2+} by the dye Fura-2 (30 min preincubation with culture at 37°C at 3 μM concentration). (a) and (b) are representative of 9 experiments performed on separate tissues from 8 patients. Mean basal $[Ca^{2+}]_i$ = 70 ± 5 nM, maximum mean change in $[Ca^{2+}]_i$ = 739 ± 171 nM.

rather than $MgATP^{2-}$, the ionic form more prevalent under most physiological conditions. The EC_{50} value of bradykinin, another agent which increases $[Ca^{2+}]_i$ in human nasal epithelial cells via a non-purinoceptor (Paradiso *et al.*, 1989), was not altered by varying $[Mg^{2+}]$ in the extracellular medium.

Cystic fibrosis nasal epithelium

Availability of CF tissues is limited, and our investigation of effects of nucleotides was restricted to examining regulation of Cl^- secretion rates and $[Ca^{2+}]_i$ levels by ATP and UTP. Only small changes in Cl^- secretion (amiloride-resistant I_{sc}) were observed in CF tissues following basolateral addition of ATP (14 ± 3 maximum mean % change in I_{sc}, n = 6) compared with normal tissues (51 ± 8 maximum mean % change in I_{sc}, n = 6). Apical administration of ATP following blockade of Na^+ absorption with amiloride resulted in two distinct patterns of response in tissues from CF subjects (Figures 8 and 9). Most tissues exhibited an increase in I_{sc} over basal levels after ATP (Figure 8a), suggesting stimulation of Cl^- secretion. However, approximately 30% of CF tissues tested responded with a change to opposite polarity of I_{sc} following ATP (Figure 8b), suggesting a secretion dominated by cations in these preparations (Bean & Friel, 1990). UTP applied to the apical surface routinely stimulated increases in Cl^- secretion in CF tissues (Figure 8c). Mean peak change in I_{sc} from post-amiloride basal levels in response to ATP or UTP application was measured in individual CF tissues to obtain the concentration-effect relationships illustrated in Figure 9.

Increases in $[Ca^{2+}]_i$ in CF tissues were also observed after addition of ATP or UTP (Figure 10). The similar potency and effectiveness of ATP and UTP in these tissues suggests the presence of a P_2 receptor type sensitive to UTP on CF epithelium.

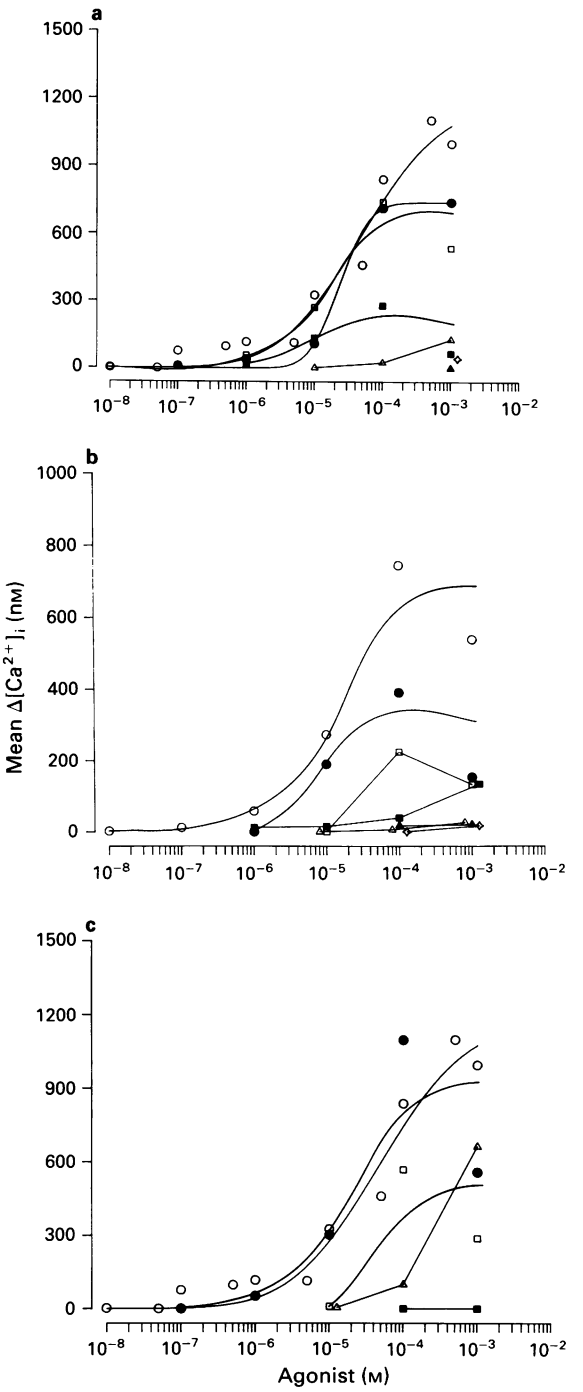

Figure 7 Log concentration-effect relationships of purine and pyrimidine compounds on $[Ca^{2+}]_i$ (mean change in $[Ca^{2+}]_i$ over basal levels). (a) comparison of agonists which bind to P_{2x}, P_{2y} or UTP-sensitive receptors, mean basal $[Ca^{2+}]_i$ in each set of experiments were (in nM): UTP (○), 81 ± 8, range 48–113, n = 5 (n = 3–6 at each agonist concentraion); ATPγS (●), 116 ± 11, range 85–151, n = 4 (n = 3–6); ATP (□), 61 ± 3, range 51–70, n = 8 (n = 3–8); 2MeSATP (■), 123 ± 19, range 95–180, n = 3 (n = 3); ADPβS (△), 91 ± 17, range 74–107, n = 3 (n = 3); αβMeATP (◇), 82 ± 16, range 50–99, n = 3 (n = 3); βγMeATP (▲), 68 ± 4, range 61–74, n = 3 (n = 3). (b) Comparison of other purine agonists with response stimulated by ATP (○). Mean basal $[Ca^{2+}]_i$ in each set of experiments were (nM): GTP (●), 117 ± 13, range 92–153, n = 3 (n = 3); AMPPNP (□), 137 ± 50, range 74–235, n = 3 (n = 3); ITP (■), 103 ± 10, range 85–121, n = 3 (n = 3); ADP (△), 94 ± 16, range 75–127, n = 3 (n = 2–3); AMP (▲), 111 ± 28, range 69–189, n = 3 (n = 3); ATPαS (◇), 89 ± 2, range 87–91, n = 1 (n = 2). (c) Comparison of UTP (○) with other pyrimidine agonists, mean basal $[Ca^{2+}]_i$ (in nM): 5BrUTP (●), 110 ± 13, range 82–152, n = 3 (n = 2–3); UDP (□), 89 ± 2, range 84–92, n = 3 (n = 3); CTP (△), 83 ± 10, range 69–102, n = 3 (n = 3); UMP (■), 78 ± 14, range 64–91, n = 2 (n = 2). S.e.mean of each data point is ⩽12% of the normalized maximum response.

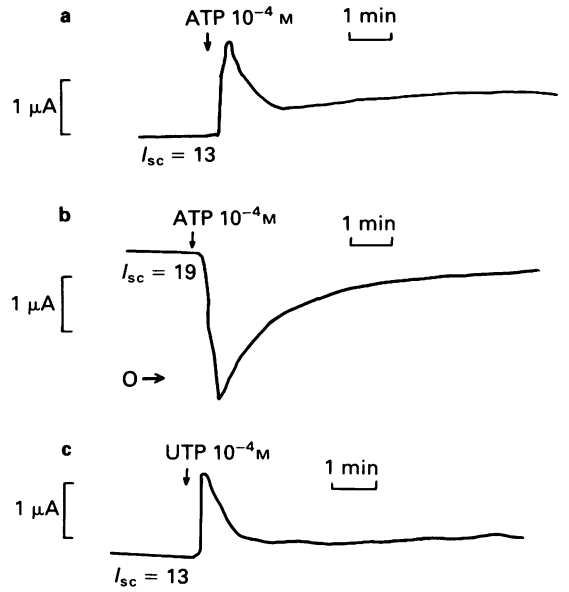

Figure 8 Representative bioelectric tracings of effect on I_{sc} of extracellular ATP (10^{-4} M) or UTP (10^{-4} M) applied to the apical surface of amiloride-pretreated (10^{-4} M) CF human nasal epithelium. (a) Cl^- secretion in response to ATP (post-amiloride $I_{sc} = 13\,\mu A\,cm^{-2}$). (b) I_{sc} response to ATP of opposite polarity (post-amiloride $I_{sc} = 19\,\mu A\,cm^{-2}$). (c) Cl^- secretory response to UTP (post-amiloride $I_{sc} = 5\,\mu A\,cm^{-2}$).

Discussion

We have demonstrated that ATP modulates ion transport rates when added to either the apical or basolateral surface of human airway epithelium. These effects appear to be mediated by cell surface receptors that respond to ATP by regulating ion transport rates, which may be related to release of Ca^{2+} from internal stores and extracellular Ca^{2+} influx.

The ion transport activities measured in this study are believed to be important in regulation of the volume and composition of secretions produced by human airway cells (Yankaskas *et al.*, 1985). Inflammatory mediators like bradykinin and histamine probably alter the water content of luminal secretions through their effects on ion flow and thus affect the rate of clearance of these secretions within the airways. Our characterization of the effects of nucleotide receptors on human nasal epithelial transport rates has led to insights into the functional effects of activation of these receptors on the metabolism of airway secretions. For example, ATP applied to the basolateral surface of the epithelium induces a large increase in ion transport rate (I_{sc}), reflecting a component of increased absorption of Na^+ in response to ATP. The increase in Na^+ absorption results in increased water movement across the epithelium, likely acting to deplete the volume of airway surface liquid. Amiloride blocks Na^+ absorption and stimulates Cl^- secretion in human nasal epithelium, probably reversing the direction of water flow. Consequently, addition of ATP to the luminal surface of the epithelium during amiloride treatment would probably accelerate this rate of Cl^- secretion and water movement.

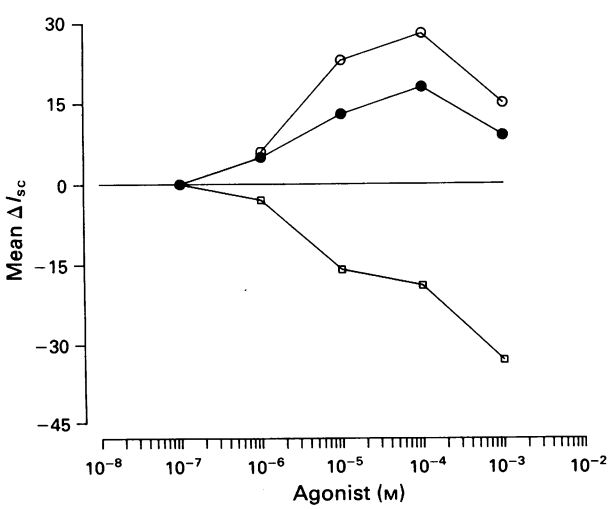

Figure 9 Log concentration-effect curves for changes in I_{sc} from basal levels when ATP or UTP are applied to the apical surface of amiloride-pretreated (10^{-4} M) cystic fibrosis tissues. Mean post-amiloride I_{sc} in each set of experiments were (in $\mu A\,cm^{-2}$): ATP [Group A (●), Group B (□)], 13 ± 1, range 3–31, $n = 4$ ($n = 4$–13 at each agonist concentration); UTP (○), 12 ± 2, range 8–14, $n = 3$ ($n = 3$). S.e.mean of each data point is ⩽13% of the normalized maximum response.

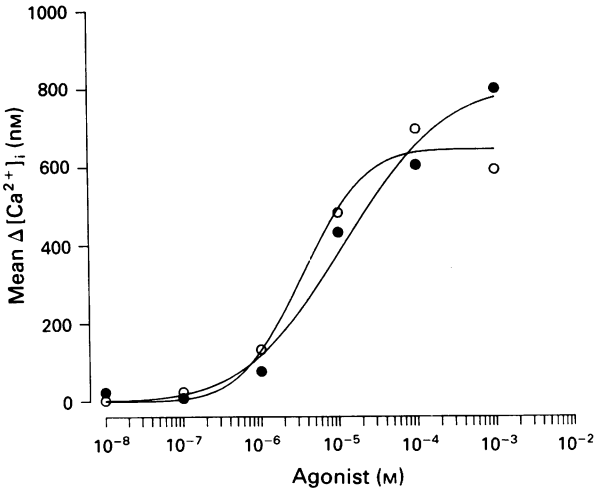

Figure 10 Log concentration-effect relationships of the effect of ATP and UTP on $[Ca^{2+}]_i$ (change from basal levels) in single cystic fibrosis nasal epithelial cells. Mean basal $[Ca^{2+}]_i$ (in nM): ATP (○), 65 ± 6, range 44–86, $n = 3$ ($n = 3$ at each agonist concentration); UTP (●), 67 ± 3, range 56–79, $n = 3$ ($n = 3$–4). S.e.mean of each data point is ⩽8% of the normalized maximum response.

Our ion transport data suggest the presence of at least one of the several reported P_2 receptor subtypes (Burnstock & Kennedy, 1985) on both the apical and basolateral membrane. The receptor on either membrane that initiates the ion transport responses is clearly not of the P_{2x} subtype as basal I_{sc} shows little or no change in response to the potent P_{2x} agonists $\alpha\beta$MeATP and $\beta\gamma$MeATP. The rank orders of potency and efficacy for agonists applied to the apical surface of human nasal epithelium were: ATP ⩾ UTP > ATPγS > 2MeSATP > ADPβS. This relationship is suggestive of the presence of a receptor sensitive to UTP and not of the typical P_{2y} subtype, which interacts most potently with 2MeSATP and ADPβS. A putative P_2 receptor responding with approximately equal potency to UTP and ATP has been reported in a number of cell types, including human neutrophils (Seifert *et al.*, 1989; Cockcroft & Stutchfield, 1989; Kuroki & Minakami, 1989), HL60 cells (Cockcroft & Stutchfield, 1989), fibroblasts (Fine *et al.*, 1989), amnion cells (Vander Kooy *et al.*, 1989), Ehrlich ascites tumour cells (Dubyak & De Young, 1985), pituitary cells (Davidson *et al.*, 1990), A431 epidermoid carcinoma cells (Hosoi & Edidin, 1989), J774 mouse macrophage cells (Greenberg *et al.*, 1988) and perfused rat liver (Haeussinger *et al.*, 1988). Controversy exists at present about whether UTP responsiveness implies the existence of a separate pyrimidine receptor (Haeussinger *et al.*, 1987; von Kuegelgen *et al.*, 1987; Seifert & Schultz, 1989; Stutchfield & Cockcroft, 1990; Pfeilschifter, 1990).

Rank orders of potency of nucleotides added to the basolateral membrane suggest that the P_2 receptor(s) found there are

also not of the P_{2x} or P_{2y} subtype, and are sensitive to UTP. The I_{sc} rank order of potency (2MeSATP > UTP > ATP > ATPγS > ADPβS) for nucleotide additions to the basolateral membrane does raise the possibility that two receptor subtypes may be required to account for the observed functional responses. The potency of the prototype P_{2y} receptor agonist, 2MeSATP, was greater than that of the other agonists tested. However, the lack of effect of another classical P_{2y} agonist, ADPβS, compared to 2MeSATP in human nasal epithelium makes it unlikely that the receptor mediating I_{sc} response is the usual P_{2y} receptor subtype. No currently accepted P_2 receptor subtype fits this potency profile. Additive studies with 2MeSATP and UTP suggest that two receptor subtypes rather than a single receptor may be required to account for this profile. Similar studies on the apical membrane did not suggest the presence of two receptor subtypes, but the failure to detect additivity could reflect limitations of the maximal response measured in this assay (Cl^- secretion), as compared to that of basolateral addition (Na^+ absorption). Rigorous identification of the presence of more than one P_2 receptor subtype on either membrane of human nasal epithelium will require antagonist compounds not currently available.

The concentration-effect relationship of potent agonists added to the basolateral membrane was biphasic in character when the I_{sc} response to 10^{-3} M drug is considered (Figure 4). However, in experiments in which ATP was added to the basolateral surface in the presence of amiloride, the relationship was sigmoidal (Figure 3), suggesting that a process that regulates the rate of Na^+ absorption is responsible for the large increment of I_{sc} at millimolar drug concentrations. Because increased Na^+ absorption is probably the transport process that contributes to the change in I_{sc} at lower nucleotide concentrations, the biphasic response may indicate linkage of purinoceptors to two effector systems that regulate Na^+ transport rates.

As in the I_{sc} studies, changes in cell $[Ca^{2+}]_i$ induced by nucleotides cannot be explained by a P_2 receptor of the P_{2x} subtype. Rank orders of potency of agonists (UTP ≥ ATP > ATPγS > 2MeSATP > ADPβS) suggest the presence of a UTP-sensitive receptor. Sensitivity of this receptor to the presence of Mg^{2+} ions suggests that it is activated by the free acid form ATP^{4-}. It is not possible to speculate about the nature of receptors on the apical and basolateral membranes because agonists may have access to both sides of cells grown on coverslips. Ongoing studies of polarized epithelial preparations specially configured for fluorescent $[Ca^{2+}]_i$ measurements should help to elucidate the receptor type(s) present on each membrane.

The ion transport and $[Ca^{2+}]_i$ data suggest that human airway epithelium responds to various extracellular nucleotides via a receptor-mediated mechanism. By analogy to other epithelia, this mechanism probably involves phospholipase C-mediated breakdown of membrane phospholipids to inositol phosphates, coupled with release of Ca^{2+} from internal stores. Preliminary studies in normal primary human nasal epithelium and CF cell lines in which inositol phosphate production in response to nucleotides was measured, support phospholipid breakdown as the mediator of calcium release (H. Alex Brown, personal communication). The similar EC_{50}s for the I_{sc} response and Ca^{2+} response to various nucleotide agonists also support a link between these two phenomena.

The effectiveness of extracellular nucleotides in stimulating Cl^- secretion by amiloride-pretreated normal human airway epithelium raises the question of therapeutic uses for these compounds in diseases of epithelial dysfunction like cystic fibrosis. Although regulatory pathways that involve cyclic AMP and Ca^{2+} phospholipid-dependent protein kinases are defective in CF airway epithelium (Boucher *et al.*, 1986), a recent report indicates that Ca^{2+}-dependent Cl^- secretory regulatory mechanisms are retained by CF epithelia (Boucher *et al.*, 1989). Our data show $[Ca^{2+}]_i$ was increased and Cl^- secretion stimulated by ATP and UTP in CF airway epithelial cells pretreated with amiloride. In a recent pilot study, amiloride administered by the aerosol route was reported to slow but not abolish the progression of CF lung disease (Knowles *et al.*, 1990). The mode of action is probably via a reduction of the excessive rate of Na^+ absorption that also characterizes this disease and, consequently, conservation of liquid on the airway surface. ATP or UTP combined with amiloride and administered to target the airway epithelium might act to induce Cl^- secretion toward the airway lumen, further increasing the water content of secretions and increasing their rate of clearance from the respiratory system. Thus, by combining purinoceptor agonists as a new class of therapeutic agents with amiloride, it may be possible to abolish the loss of lung function consequent to this disease.

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