function of CFTR. In addition, using this method, it is now possible to compare the turnover of CFTR in the membrane of cells from CF patients with different genotypes, thus, providing new insights concerning the effects of different mutations on CFTR.

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Extracellular ATP and UTP Induce Chloride Secretion in Nasal Epithelia of Cystic Fibrosis Patients and Normal Subjects in vivo*

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Airway epithelia of cystic fibrosis (CF) patients absorb excessive sodium and have defective regulation of the apical membrane chloride channel. Amiloride, a sodium channel blocker, shows promise for treating CF lung disease by inhibiting sodium absorption, improving mucociliary clearance, and slowing the rate of decline of vital capacity when administered as an aerosol following antibiotic therapy. Recent in vitro data indicate that triphosphate nucleotides (ATP and UTP) interact with extracellular purinergic (P2) receptors and initiate chloride secretion in human airway epithelia. Because there are no available therapeutic agents to improve chloride secretion in airways of CF patients, we tested whether nucleotides were effective chloride secretagogues in vivo. Since extracellular nucleotides were more effective in inducing chloride secretion in CF than normal subjects, we performed microelectrode studies in primary cultures of nasal epithelia to quantitate the rates of chloride secretion and define the mechanism of action.

METHODS

Nine normal subjects (6 F, 3 M; age 18 to 28 years) and 12 CF patients (7 F, 5 M; age 19 to 38 years) were studied. The same 5 normal subjects and 5 CF patients were studied at all concentrations in the dose-response studies. Specimens for cell culture were obtained from nasal tissue excised from 5 CF patients and 4 normal subjects undergoing clinically indicated operations. The nasal potential difference (PD) and response to superfusion with amiloride and/or chloride-free solution and/or nucleotide (or nucleoside) was measured. Double-barrelled chloride-selective microelectrodes were used to assess the driving force for chloride flow across the apical membrane, and the absolute permeability of apical membrane to chloride was calculated before and during the addition of nucleotides.

RESULTS

Effect of Extracellular ATP and UTP on Chloride Secretion in vivo

After amiloride (10-4 M) pretreatment, superfusion of ATP and UTP in log-incremental doses (10-7 to 10-3 M) induced hyperpolarization of the nasal epithelium of normal subjects and CF patients in a manner consistent with chloride secretion. The effect of ATP and UTP was dose-related in CF and normal subjects, and the nucleotides were equipotent in both groups of subjects (K0.5 = 2 - 5 x 10-4 M; maximal effective concentration ~ 10-4 M). However, the efficacy of both ATP and UTP was greater in CF patients (ΔPD = 19.8 ± 1.4 and ~ 15.0 ± 1.7 mV) than in normal subjects (ΔPD = 6.9 ± 0.6 and ~ 8.1 ± 0.9 mV).

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34th Annual Thomas L. Petty Aspen Lung Conference
Superfusion of ATP (10⁻⁴ M) and UTP (10⁻⁴ M) onto nasal epithelium of 3 normal subjects without amiloride pretreatment had no effect on PD or chloride secretion (ΔPD = −0.5 ± 0.8 and −2.3 ± 2.0 mV, respectively). The lack of effect of nucleotides in normal subjects in the absence of amiloride reflects the fact that chloride is in electrochemical equilibrium across the apical membrane in the baseline state, so that activation of apical chloride channels is not associated with ion flow. In contrast, superfusion of ATP and UTP onto the nasal epithelium of 4 CF patients without amiloride pretreatment induced a depolarization of the PD (ΔPD = +8.8 ± 2.1 and +5.8 ± 1.8 mV, respectively). The response in CF patients reflects the fact that chloride is below electrochemical equilibrium across the apical membrane in the baseline state; activation of chloride channels without amiloride pretreatment likely leads to chloride influx (absorption) and a decrease in the PD.

**Time Course of Nucleotide Effects on Chloride Secretion**

A hallmark of the nucleotide receptor in vitro is the equipotency of ATP and UTP initiating chloride secretion. We compared ATP and UTP in amiloride-pretreated nasal epithelium in vitro by measuring first, the time to reach the maximal change in PD in response to superfusion of maximal concentrations of nucleotide (10⁻⁴ M – 10⁻³ M); and second, the duration of nucleotide effect. Both nucleotides induced hyperpolarization of the PD across amiloride-pretreated nasal epithelium more rapidly in CF (ATP = 47.5 ± 10.8 sn = 7; UTP = 43.2 ± 11.9 sn = 6) than in normal subjects (ATP = 128.7 ± 16.0 sn = 7; UTP = 132.6 ± 7.9 sn = 5). The same pattern of response was noted for both nucleotides during superfusion with amiloride and chloride-free solution, i.e., ATP responded more rapidly (CF: ATP = 47.2 ± 11.0 sn = 7; UTP = 48.0 ± 25.7 sn = 3; normal: ATP 163.4 ± 29.5 sn = 7; and UTP 132.5 ± 30.0 sn = 3). The persistence of the PD response (% of maximal response persisting at 6 min) during continuous superfusion of maximal concentrations of nucleotide was similar for ATP and UTP in amiloride-pretreated nasal epithelium of 5 CF patients (59.1 ± 3.9 and 67.8 ± 4.0%) and 5 normal subjects (43.5 ± 10.9 and 52.4 ± 6.5%).

**Effect of Adenosine and Uridine on Chloride Secretion in vitro**

We tested the effect of adenosine and uridine (breakdown products of ATP and UTP, respectively) on chloride secretion in vitro because these nucleosides could act via P₁ receptors. Adenosine (10⁻⁴ M) superfusion onto amiloride (10⁻⁴ M) pretreated nasal epithelium of 5 normal subjects increased the PD (ΔPD = −4.0 ± 1.3 mV); the sequential addition of ATP (10⁻⁴ M) further increased the PD (ΔPD = −4.4 ± 0.4 mV) (Fig 1). Adenosine was ineffective in 3 CF patients (ΔPD 0.0 ± 1.2 mV), but the sequential addition of ATP (10⁻⁴ M) induced a marked increase in the PD (ΔPD = −17.0 ± 3.1 mV) (Fig 1). Uridine (10⁻⁴ M) had no effect on 3 CF or 3 normal subjects.

**Mechanism of Nucleotide-Induced Chloride Secretion in vitro and in vivo**

Primary cultures of CF and normal nasal epithelia were studied with double-barreled Cl⁻-selective microelectrodes to assess the mechanism of action of UTP to induce chloride secretion. The tissues were pretreated with amiloride to generate driving forces favorable for chloride secretion. As seen in Table 1, the residual ion current (equivalent short circuit current; I₁eq) that is measured in amiloride-treated normal human nasal epithelia is an index of the net chloride secretory flow. The relatively large residual current (I₁eq) after amiloride in the normal cells reflects, in part, the basal activation of the apical membrane chloride conductance. In contrast, the CF cells are relatively impermeable to chloride in the baseline state, and the rate of chloride secretion (residual I₁eq after amiloride) is close to zero despite the fact that the electrochemical driving force for chloride exit across the apical membrane is larger in CF than in normal cells. Following UTP, the maximal increase in I₁eq of normal and CF epithelia is associated with cellular changes that indicate activation of the apical membrane CF conductance, i.e., depolarization of Va and decreased resistance (decreased Rt and fha) of the apical membrane. The changes in I₁eq and PD, are larger in CF than in normal preparations, which parallels the larger change in PD induced by UTP in vivo. The larger change in chloride current in CF reflects the fact that the current before UTP is smaller in CF than in normal cells, whereas the maximally stimulated chloride currents are similar. Because the electrochemical driving force for chloride exit during the maximal secretory response to UTP is not different between CF and normal cells (−8 mV), and because the chloride currents are similar during UTP the maximal activation of the apical membrane chloride conductance is similar in CF and normal nasal epithelia. Direct calculation of the apical membrane chloride permeability (PaCl), using an equivalent circuit analysis, was consistent with this notion.

The mechanisms of nucleotide effects were also studied in nasal epithelia of CF and normal subjects in vitro. In these studies, the chloride concentration of the amiloride-containing perfusate was reduced to zero to generate a large

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**Figure 1.** Effect of the P₁ agonist, adenosine (ADO; hatched bars) and the P₂, agonist, ATP (open bars) on PD of amiloride pretreated nasal epithelium of normal subjects and CF patients. Superfusion of ATP (10⁻⁴ M) alone induced greater CF secretion in 8 CF patients than in 7 normal subjects. In 5 normal subjects, ADO (10⁻⁴ M) induced CF secretion, and the addition of ATP (10⁻⁴ M) further increased CF secretion. In 3 CF patients, ADO had no effect, but addition of ATP induced a CF secretory response. Asterisk indicates different from ADO in normal subjects p<0.05 (paired Students t-test).
Table 1—Effect of UTP on Bioelectrical Parameters and Intracellular Cl\(^-\) Activities of Cultured Normal and CF Nasal Epithelial Cells Pretreated with Amiloride

<table>
<thead>
<tr>
<th></th>
<th>PDᵢ, mV</th>
<th>Vᵢ, mV</th>
<th>Vₑ, mV</th>
<th>Rₑ, Ω cm²</th>
<th>Iₑq, µA/cm²</th>
<th>fRₑ</th>
<th>aCl, mM</th>
<th>ΔFᵢaCl, mV</th>
<th>PaCl, 1 × 10⁻⁴ cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>-2.6 ± 0.4</td>
<td>-30.9 ± 2.6</td>
<td>-33.5 ± 2.6</td>
<td>268 ± 50</td>
<td>-11.2 ± 2.0</td>
<td>0.56 ± 0.04</td>
<td>42.4 ± 2.8</td>
<td>10.6 ± 1.6</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>UTP</td>
<td>-4.9 ± 0.5†</td>
<td>-23.8 ± 2.9†</td>
<td>-37.7 ± 2.9†</td>
<td>215 ± 35†</td>
<td>-25.7 ± 2.8†</td>
<td>0.41 ± 0.05†</td>
<td>45.9 ± 1.3†</td>
<td>7.7 ± 2.2†</td>
<td>12.5 ± 2.4†</td>
</tr>
<tr>
<td>Δ (UTP-Control)</td>
<td>-2.3 ± 0.3</td>
<td>7.1 ± 1.0</td>
<td>4.8 ± 1.0</td>
<td>-53 ± 16</td>
<td>-14.5 ± 1.6</td>
<td>-0.15 ± 0.02</td>
<td>7.1 ± 1.2</td>
<td>-2.9 ± 0.9</td>
<td>8.2 ± 2.2</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td></td>
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<tr>
<td>Control</td>
<td>-0.8 ± 0.3</td>
<td>-41.9 ± 3.5</td>
<td>-42.7 ± 3.5</td>
<td>420 ± 78</td>
<td>-2.2 ± 0.9</td>
<td>0.76 ± 0.03</td>
<td>37.3 ± 3.3</td>
<td>17.7 ± 3.4</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>UTP</td>
<td>-7.3 ± 1.3†</td>
<td>-26.9 ± 3.6†</td>
<td>-34.2 ± 2.8†</td>
<td>281 ± 47†</td>
<td>-31.7 ± 7.1†</td>
<td>0.43 ± 0.04†</td>
<td>45.3 ± 4.0†</td>
<td>8.0 ± 2.4†</td>
<td>12.4 ± 3.1†</td>
</tr>
<tr>
<td>Δ (UTP-Control)</td>
<td>-6.5 ± 1.1†</td>
<td>15.0 ± 2.7†</td>
<td>8.5 ± 2.3</td>
<td>-139 ± 34†</td>
<td>-29.5 ± 7.2†</td>
<td>-0.33 ± 0.03†</td>
<td>8.0 ± 1.9</td>
<td>-9.7 ± 2.3†</td>
<td>11.7 ± 3.0</td>
</tr>
</tbody>
</table>

*Values represent means SEM; n = 9 for normal, n = 10 for CF. Control and UTP data from a continuous impalement for each tissue. All UTP parameters were recorded simultaneously with the maximal change in PD. Amiloride added to luminal bath at 10⁻⁴ M; UTP added to luminal bath at 10⁻⁴ M. UTP values were not significantly different for normal vs CF (unpaired Student's t-test).

PD, is transepithelial potential difference; Vᵢ, Vₑ, potential across the apical and basolateral membranes, respectively; Rₑ, transepithelial resistance; Iₑq, equivalent short-circuit current; fRₑ, fractional resistance of the apical membrane; aCl, intracellular Cl⁻ activity; ΔFᵢaCl, electrochemical driving force for Cl⁻ movement across apical membrane. Positive values indicate ΔFᵢaCl is directed from cell to lumen; and PaCl, calculated CF permeability of the apical membrane.

†Different from control value p < 0.05 (paired Student's t-test).

Different from normal Δ (UTP-control) p < 0.05 (unpaired Student's t-test).

different chemical gradient for chloride across the apical membrane and paracellular path; activation of apical membrane chloride channels by ATP or UTP would be associated with an increase in PD. Superfusion of ATP (10⁻⁴ M) and UTP (10⁻⁴ M) in the presence of an amiloride- and chloride-free solution induced a greater increase in UTP (ΔPD = 33.6 ± 2.0 mV, n = 8 and -39.8 ± 4.4 mV, n = 5, respectively) than in normal subjects (ΔPD = 20.6 ± 1.7 mV, n = 7 and -21.2 ± 3.2 mV, n = 3, respectively). The absolute magnitude of the PDs after superfusion of ATP or UTP was similar in CF and normal subjects (~50 to -54 mV), suggesting an equally effective total activation of apical membrane chloride conductance in both groups.

**Discussion**

Extracellular nucleotides are effective chloride secretagogues in both CF and normal subjects when applied to the ciliated surface of nasal epithelia in vivo. The equipotency of ATP and UTP to induce chloride secretion suggests the response is mediated by nucleotide receptors, a subclass of P₂ purergic receptors.4,11,12

The effect of adenosine to induce chloride secretion in nasal epithelia of normal subjects, additive with responses to ATP, suggests the presence of both P₁ and P₂ receptors in airway epithelia.11 The absence of a response to adenosine in CF patients is consistent with the defect in cAMP-mediated chloride secretion in CF,4 and indicates the ATP activation of chloride secretion is mediated through P₂ receptors in CF patients.

The baseline permeability of the apical membrane to chloride is lower in CF compared to normal subjects.7,10 The greater effect of UTP to induce chloride secretion in CF patients is associated with a greater change in the apical membrane chloride conductance, and reflects recovery of a basal chloride permeability in CF, as well as stimulation of chloride secretion to the same maximal level in both groups. This may reflect activation of the defective chloride transport path in CF, or activation of another population of chloride channels with the same secretory capacity.

The time course of nucleotide activation of chloride secretion is intriguing in 2 regards. First, the more rapid activation by ATP and UTP of maximal chloride secretion in CF suggests a difference in access of nucleotides to the receptor, or a difference in signalling or effector mechanisms between CF and normal epithelia. The present data do not address those considerations. Second, the relatively prolonged duration of effect during continuous superfusion suggests a link between nucleotide receptors and chloride channels in addition to the receptor- and inositol-mediated increase in cytosolic calcium,13 which is usually associated with a short-lived response.14 The relationship between the nucleotide receptor and the type of chloride channel is unknown.

This study suggests that pharmacologic induction of chloride secretion may provide a new approach to the treatment of CF airways disease. However, safety of aerosolized nucleotides must be better defined, particularly regarding bronchoconstriction15 and effects on secretory and inflammatory cells in the respiratory tract.16,17 A combination of amiloride (or another sodium channel blocker) and nucleotide will probably be required to achieve effective secretion of chloride (and water) into the airway lumen.

ACKNOWLEDGMENTS: We would like to thank Joseph Robinson, M.Sc., for technical assistance and L. Brown for editorial assistance.

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Effects of Endothelin-1 on Tracheal Submucosal Gland Secretion and Epithelial Function in the Ferret*

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Endothelin-1 (ET-1) is one of a family of novel peptides recently discovered in the human genome. It was originally isolated from cultured porcine aortic endothelial cells and is a potent constrictor of isolated blood vessels. ET-1 is also the most potent of the endothelins at contracting bronchial smooth muscle.

Immunofluorescent staining for ET-1 has been found in epithelial cells from the hilum to the periphery of the conducting airways of mice, and epithelial cells from human bronchii form and release endothelin-like material. However, the effects of ET-1 on airway mucosal tissues, including submucosal glands and the epithelium itself, have not been studied.

The ferret trachea in vitro is a preparation which allows the simultaneous measurement of a number of airway functions, and we have used this preparation to assess the activity of ET-1 on baseline smooth muscle tone, submucosal gland secretion (lysozyme secretion from serous cells), and epithelial function and integrity (active epithelial albumin transport and transepithelial potential difference [PD]). We have also examined the effect of ET-1 on responses to the muscarinic agonist methacholine and the α-adrenoceptor agonist, phenylephrine. The contraction of bronchial smooth muscle by ET-1 may be mediated through activation of dihydropridine-sensitive Ca⁺⁺ channels. The role of these Ca⁺⁺ channels in the responses to ET-1 in the present study was assessed using the Ca⁺⁺ channel blocker, nifedipine.

METHODS

The trachea was removed from the ferret, cannulated at both ends, and mounted laryngeal end downwards in an organ bath. The trachea was bathed on the submucosal side with Krebs buffer...