UTP regulation of ion transport in alveolar epithelial cells involves distinct mechanisms

Chuanxiu Yang, Lijing Su, Yang Wang, and Lin Liu
Lundberg-Kienlen Lung Biology and Toxicology Laboratory, Department of Physiological Sciences, Oklahoma State University, Stillwater, Oklahoma

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Yang C, Su L, Wang Y, Liu L. UTP regulation of ion transport in alveolar epithelial cells involves distinct mechanisms. Am J Physiol Lung Cell Mol Physiol 297: L439–L454, 2009. First published June 19, 2009; doi:10.1152/ajplung.90268.2008.—UTP is known to regulate alveolar fluid clearance. However, the relative contribution of alveolar type I cells and type II cells to this process is unknown. In this study, we investigated the effects of UTP on ion transport in type I-like cell (AEC I) and type II-like cell (AEC II) monolayers. Luminal treatment of cell monolayers with UTP increased short-circuit current ($I_{sc}$) of AEC II but decreased $I_{sc}$ of AEC I. The Cl$^-$ channel blockers NPPB and DIDS inhibited the UTP-induced changes in $I_{sc}$ ($\Delta I_{sc}$) in both types of cells. Amiloride, an inhibitor of epithelial Na$^+$ channels (ENaC), abolished the UTP-induced $\Delta I_{sc}$ in AEC I, but not in AEC II. The general blocker of K$^+$ channels, BaCl$_2$, eliminated the UTP-induced $\Delta I_{sc}$ in AEC II, but not in AEC I. The intermediate conductance (IKca) blocker, clofilium, also blocked the UTP effect in AEC II. The signal transduction pathways mediated by UTP were the same in AEC I and AEC II. Furthermore, UTP increased CI$^-$ secretion in AEC II and CI$^-$ absorption in AEC I. Our results suggest that UTP induces opposite changes in $I_{sc}$ in AEC I and AEC II, likely due to the reversed Cl$^-$ flux and different contributions of ENaC and IKca. Our results further imply a new concept that type II cells contribute to UTP-induced fluid secretion and type I cells contribute to UTP-induced fluid absorption in alveoli.

short-circuit current; fluid transport

ALVEOLAR EPITHELIAL CELLS can be divided into two groups: alveolar epithelial type I cells and alveolar epithelial type II cells. These cells cover ~99% of the internal surface of the lung. In the fetal lung, the alveolar space is filled with liquid. Just before and shortly after birth, lung switches from fluid secretion to absorption. The liquid in the alveolar space is cleared, and the lung begins to perform gas exchange. In the adult lung, there is only a very thin layer of liquid lining the apical side of alveolar epithelia. Efficient gas exchange depends on a proper amount of alveolar lining fluid (50).

It is generally accepted that fluid transport in alveoli is an active process. It is controlled by ion channels and transporters, which are located in the cell membranes of alveolar epithelia. Various ion channels and transporters have been identified in the apical or basolateral membranes of type II cells, including epithelial sodium channels (ENaC) (21), cyclic fibrosis transmembrane regulator (CFTR) (6), and Na$^+$-K$^+$-ATPase (65). In the past, no functional ion channels have been confidently detected in type I cells except water channels (14, 54). This leads to the concept that type II cells govern fluid clearance and type I cells only provide a route for water absorption. However, several recent reports have convincingly demonstrated that type I cells contain functional ion channels and transporters that regulate fluid transport. The results from immunohistochemical and immunoelectron microscopy studies have revealed that the ENaC $\alpha_1$, $\beta_1$, $\gamma$-subunits and the Na$^+$-K$^+$-ATPase $\alpha_1\gamma_2\delta$-subunits are present in type I cells (4, 34, 61). Those results have also been confirmed by quantitative PCR and Northern blot. Most recently, patch-clamp studies have shown that type I cells contain functional ENaC, pimozide-sensitive cation channels, K$^+$ channels, and CFTR (33). Type I cells display a linear $^{22}$Na$^+$ uptake over time, which is inhibited by amiloride, an ENaC inhibitor. Surprisingly, type I cells take up 2.5 times more Na$^+$ per milligram of protein than type II cells (34). It has been estimated that up to 60% of the fluid clearance is governed by type I cells (61). All of these studies clearly suggest that type I cells play a role in active alveolar fluid transport. However, the relative contribution of type I cells and type II cells to this process is still unknown.

In addition to its central role in many biochemical processes, UTP is also an important extracellular molecule that regulates a broad spectrum of cell functions via the activation of P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors (40). UTP regulates transepithelial ion transport in airway (13, 48), pancreatic ducts (25), cortical collecting duct (43), and distal colonic mucosa and renal inner medullary collecting ducts (51). Recently, much attention has been paid on the regulation of UTP on airway epithelial ion transport. In primary cultures of human nasal epithelial cells, UTP levels in the thin (1 $\mu$m) film of surface liquid layer approaches threshold values for P2Y receptor activation, indicating that constitutive release of UTP may regulate “baseline functions” in the airways by binding P2Y$_2$ and P2Y$_6$ receptors (41). UTP activates the Ca$^{2+}$-activated Cl$^-$ channel (CaCC) primarily via the P2Y$_2$ receptor and partially reverses the abnormal salt and water secretion in cystic fibrosis (CF) by a pathway bypassing the CFTR (48). Although P2Y$_2$ receptors are expressed in alveolar epithelial cells (11, 60), only sporadic studies focus on the effects of UTP on alveolar epithelial ion transport. In primary cultures of human nasal epithelial cells, UTP inhibits ENaC activity and decreases alveolar fluid clearance (12, 22, 26, 59) by an unknown mechanism. In addition, respiratory syncytial virus infection of the bronchoalveolar epithelia inhibits alveolar fluid clearance by inducing UTP release into the alveolar space (12). Therefore, UTP control of luminal electrolyte transport and clearance of alveolar fluid may provide important mechanisms to the homeostasis of the alveolar surface liquid layer under physiological and pathological conditions. Furthermore, UTP has been advocated as the prototypical compound for aerosol therapy in CF patients (2, 36). Alveoli are certain places that are affected. Thus, detailed investigation of the UTP regulation on ion transport in alveolar epithelia is needed.

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Address for reprint requests and other correspondence: L. Liu, Dept. of Physiological Sciences, Oklahoma State Univ., 264 McElroy Hall, Stillwater, OK 74078 (e-mail: lin.liu@okstate.edu).
Although relatively pure type I cells have been isolated, the formation of type I cell monolayer on permeable supports that is suitable for measuring the electrophysiological characteristics using Ussing chamber has not been reported. In response to alveolar epithelial injury, type II cells serve as progenitor cells for type I cells (66). When they are cultured on plastic dishes or permeable inserts under a liquid-liquid condition, type II cells transdifferentiate into type I-like cells (AEC I) with expression of some type I cell markers, such as $T_{la}$ (62), aquaporin 5 (5), and caveolin-1 (7). Whereas, when they are cultured on permeable material under an air-liquid condition, type II cells maintain some of their phenotype and can be used as type II-like cells (AEC II) (49). In this study, using AEC I and AEC II, we investigated the regulation of alveolar epithelial ion channels by UTP and determined the relative contribution of type I cells and type II cells to ion transport. Our results indicate that UTP induced opposite short-circuit current ($I_{sc}$) changes in AEC I and AEC II due to different regulatory mechanisms. This leads to a novel concept that type II cells contribute to UTP-induced fluid secretion, whereas type I cells mediate UTP-induced fluid absorption. Our results should further help our understanding of the mechanisms of lung edema, injury, bacteria infection, and CF.

MATERIALS AND METHODS

Chemicals and reagents. DMEM, FBS, nonessential amino acid, penicillin, streptomycin, and M-MLV reverse transcriptase were purchased from Invitrogen (Carlsbad, CA). Pancreatic elastase was from Worthington (Lakewood, NJ). Costar snapwell 3407 inserts were from Fisher Scientific (Springfield, NJ). TRI reagent was from Mo-}

Table 1. Primers used for real-time PCR

<table>
<thead>
<tr>
<th>GenBank ID</th>
<th>Gene Name</th>
<th>Primer</th>
</tr>
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<tr>
<td>BC061754.1</td>
<td>P2Y2</td>
<td>Forward: GATACTCATCATTGTTGCTCCCAAG</td>
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<tr>
<td>NM_031828</td>
<td>Maxi-KCa</td>
<td>Forward: AGCTCTGACCTGACACACAA</td>
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<tr>
<td>NM_023021</td>
<td>IKCa</td>
<td>Forward: CAATCTGCCGGTTGAC</td>
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<td>NM_01709</td>
<td>Kir 6.1</td>
<td>Forward: AAGACGGCTGAGTAGCCAGA</td>
</tr>
<tr>
<td>AB045281.1</td>
<td>SUR2B</td>
<td>Forward: TGCCGTTGCACTGGGGGCTG</td>
</tr>
<tr>
<td>AJ133685.1</td>
<td>KvLQT1</td>
<td>Forward: TGCCGCTTCAATGTCGACCAAG</td>
</tr>
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Primers were designed with Primer Express software. All gene sequence information was obtained using nucleotide databases. P2Y2, P2Y2 receptor; Maxi-KCa, potassium large conductance calcium-activated channel; IKCa, potassium intermediate/small conductance calcium-activated channel; Kir 6.1, potassium inwardly rectifying channel; SUR2B, sulphonylurea receptor 2b; KvLQT1, KvLQT-1 potassium channel.
PCR quantification was based on the delta C_t method (57). Data were normalized with 18S rRNA.

Cell morphology and immunocytochemistry. The monolayers were fixed by 4% formaldehyde, embedded in paraffin, and transected into 4-µm slices. The slides were extracted with xylene to remove paraffin, washed, and incubated with 3% hydrogen peroxide to eliminate endogenous peroxidase. The slides were treated with 0.3% Triton X-100 to permeabilize the cell membrane and 10% FBS to block nonspecific binding sites. The immunostaining was done as previously described (53). Briefly, the slides were incubated with mouse monoclonal anti-ABCA3 or anti-T1α antibodies (both 1:200 dilution) followed by incubation with FITC-conjugated anti-mouse IgG (1:500 dilution) or Cy3-conjugated anti-mouse IgG (1:250 dilution). The slides were examined using a Nikon Eclipse E600 fluorescence microscope.

Electrophysiology. Cell monolayers were mounted in an Ussing chamber connected to a DVC-1000 voltage clamp amplifier via Ag/AgCl electrodes and 3 M KCl agar bridges. Data were acquired by the Powerlab system (ADInstruments, Colorado Springs, CO). Before each experiment, the offset of electrodes and the compensation of bath solution resistance were carefully performed by using a blank insert. The cell monolayer was first maintained under an open-circuit condition, and the transepithelial potential difference (PD) was monitored. The transepithelial voltage difference was clamped to 0 if it stabilized. The I_w were recorded continually with the software, Chart v4.0 (ADInstruments), and allowed to stabilize at least 10 min before each experiment. Changes in transepithelial resistance (R_t) were monitored by imposing a 1-s voltage pulse of 2 mV across the monolayer every 5 min. R_t was calculated by Ohm’s law. The temperature of bath solution was maintained at 37°C by a circulating water bath. The composition of the bath Ringer solution was (in mM): 115 NaCl, 5 KCl, 1.2 CaCl_2, 1.2 MgCl_2, 25 NaHCO_3, 3.3 NaH_2PO_4, 0.8 Na_2HPO_4, and 10 glucose (basolateral side) or 10 mannitol (apical side). In the ion substitution experiment, a Cl⁻-free Ringer solution was formulated by replacing Cl⁻ using equimolar gluconate. For a Na⁺-free solution, NaCl and NaHCO_3 were replaced by equimolar choline chloride and choline bicarbonate. All solutions were bubbled with 5% CO_2 and 95% O_2.

Western blot. Cells in the monolayers were lysed in lysis buffer (Pierce Rockford, IL). Protein was separated on 10% SDS-polyacrylamide gels and then transferred to a nitrocellulose membrane using the wet transfer apparatus (Bio-Rad). After being blocked with 5% nonfat milk overnight, the membranes were incubated with polyclonal anti-β-ENaC or γ-ENaC (1:300) or mouse monoclonal anti-GADPH (1:3,000) primary antibodies overnight at 4°C. Then, the membranes were washed and incubated with the goat anti-rabbit (1:2,000) or goat anti-mouse (1:3,000) primary antibodies. The membranes were washed and incubated with the goat anti-rabbit (1:2,000) or goat anti-mouse (1:2,000) secondary antibodies. Finally, the signal was developed with ECL. The quantification was performed by using Quantity One 4.0.3 software (Bio-Rad).

Unidirectional Cl⁻ transport. The Cl⁻ transport across the AEC I and AEC II monolayer was determined as previously described (42). Monolayers with transmembrane resistances within 10% of each other were selected for this experiment. Unidirectional Cl⁻ transport experiments were performed under a short-circuit condition. The transepithelial potential was held at 0. Paired monolayers were measured at the same time. One was used to measure the apical-to-basolateral Cl⁻ flux, and another one was used to measure the basolateral-to-apical Cl⁻ flux. [³⁶Cl⁻]NaCl was added to the paired monolayers at either the apical or basolateral side of the monolayer at the concentration of 1 µCi/ml. Samples were taken out from the other side of the monolayer. The monolayers were incubated with [³⁶Cl⁻]NaCl for 30 min to allow the isotope to equilibrate with the intracellular compartment. Then, two 20-min [³⁶Cl⁻] transport measurements were conducted. The first period was used to measure the basolateral Cl⁻ transport from apical side to basolateral side or from basolateral side to apical side. The second one was done in the presence of 10 µM UTP (apical side addition). [³⁶Cl⁻] radioactivity in the collections was determined by a liquid scintillation counter. The net [³⁶Cl⁻] transport was calculated by subtracting the basolateral to apical [³⁶Cl⁻] transport from the apical to basolateral [³⁶Cl⁻] transport.

RESULTS

AEC I and AEC II cell phenotypes. In this study, AEC I and AEC II were obtained by culturing primary type II cells on air-liquid and liquid-liquid models, respectively. We first confirmed these two models under our experimental conditions. Real-time PCR analysis indicated that mRNAs of SP-B and SP-C, both type II cell markers, were highly expressed in the cells cultured on the air-liquid model, but lowly expressed in the cells cultured on the liquid-liquid model. In contrast, mRNAs of aquaporin 5 and T1α, both type I cell markers, were highly expressed in the cells cultured on the liquid-liquid model (Fig. 1A). Based on the availability of suitable antibodies, we also performed immunostaining with anti-ABCA3 (a type II cell marker) and anti-T1α (a type I cell marker) antibodies. The results revealed that ABCA3 was only detected in the cells cultured on the air-liquid model, and T1α was only detected in the cells cultured on the liquid-liquid model (Fig. 1B). No signal was detected in the negative control, in which primary antibodies were omitted. These results suggest that the cells cultured on the air-liquid model maintain the type II cell phenotype, whereas the cells cultured on the liquid-liquid model transdifferentiated to AEC I. These two in vitro AEC I and AEC II models were used for the subsequent experiments.

Electrophysiological characteristics of AEC I and AEC II monolayers. The electrophysiological characteristics of AEC I and AEC II were determined by an Ussing chamber. The transmembrane resistances across AEC I and AEC II monolayers before mounting to an Ussing chamber were 1,774.3 ± 80.6 Ω-cm² (means ± SE; n = 62) and 1,570.2 ± 71.4 Ω-cm² (n = 62), respectively. The cell monolayers generated a PD of 9.27 ± 0.36 mV/cm² (n = 80) and 7.14 ± 0.40 mV/cm² (n = 80) in AEC I and AEC II, respectively. Basal 1_w of AEC I and AEC II were 7.33 ± 0.32 μA/cm² (n = 46) and 5.12 ± 0.19 μA/cm² (n = 35), respectively.

Na⁺, Cl⁻, and K⁺ channels are considered as the primary ion channels in alveolar epithelial cells to control transepithelial ion transport (50). Thus, we examined the contribution of Na⁺-, Cl⁻-, and K⁺ channels to the basal 1_w by ion substitution and treatment of the cell monolayers with antagonists. First, we tested the involvement of Na⁺ and Na⁺ channels in the basal 1_w. In all the experiments, the glucose was replaced by equimolar mannitol in apical bath solution to eliminate the activity of Na⁺-glucose cotransport. Bathing both sides of cell monolayers with Na⁺-free Ringer solution reduced the 1_w to 0.16 ± 0.06 μA/cm² (n = 7) and 0.90 ± 0.54 (n = 5) in AEC I and AEC II, respectively (Fig. 2A). Treatment of cell monolayers from the basolateral side with 200 μM ouabain, an inhibitor of Na⁺-K⁺-ATPase, decreased the 1_w to 1.41 ± 0.32 μA/cm² (n = 5) and 2.70 ± 0.13 (n = 5) in AEC I and AEC II, respectively. We also examined the sensitivity of basal 1_w to amiloride. Amiloride inhibited 1_w in a dose-dependent manner on both AEC I and AEC II monolayers with a maximal
inhibitory concentration of \( \sim 10 \) \( \mu \)M. The amiloride-sensitive part of basal \( I_{sc} \) was \( 58 \pm 3\% \) in AEC II and \( 74 \pm 2\% \) in AEC I. The \( K_{0.5} \) of amiloride was \( 633 \) nM in AEC II, whereas \( 1,109 \) nM in AEC I (Fig. 2B). Apical application of pimozide (50 \( \mu \)M), a blocker of cyclic nucleotide-gated, nonselective cation channels (CNG), had no effect on basal \( I_{sc} \) in both AEC I and AEC II in the presence or absence of amiloride. Next, we tested the contribution of \( Cl^{-} \) and \( Cl^{-} \) channels to basal \( I_{sc} \). Bathing both sides of cell monolayers with \( Cl^{-} \)-free Ringer solution reduced the \( I_{sc} \) to \( 2.56 \pm 0.22 \mu A/cm^2 \) (\( n = 5 \)) and \( 2.92 \pm 0.31 \mu A/cm^2 \) (\( n = 6 \)) in AEC I and AEC II, respectively. In the presence of 20 \( \mu \)M amiloride, apical application of 200 \( \mu \)M NPPB, a general inhibitor of \( Cl^{-} \) channels, further decreased the \( I_{sc} \) in AEC II but not in AEC I. Similar effects were observed when the monolayers were treated by 50 \( \mu \)M CFTRinh-172, an inhibitor of CFTR, from the apical side. These results indicated that there was background secretion of \( Cl^{-} \) in AEC II, but not in AEC I, which involves CFTR. Finally, we treated the cell monolayers from the basal side with 5 mM BaCl\(_2\), a general blocker of \( K^{+} \) channels. At the same time, 5 mM mannitol was added into the apical bath solution to prevent the formation of an osmotic gradient. BaCl\(_2\) reduced the basal \( I_{sc} \) (35\%) in AEC II but had little effect on AEC I, indicating that there was strong background activity of \( K^{+} \) channels only in AEC II.

Expression of ion channels and transporters in AEC I and AEC II. The results above indicated that \( Na^{+}, Cl^{-}, \) and \( K^{+} \) channels were differentially involved in basal \( I_{sc} \) in AEC I and AEC II. To determine whether the difference is due to the expression levels of ion channels or transporters, we measured mRNA expression levels of ENaC and \( K^{+} \) channels in AEC I and AEC II by real-time PCR. The mRNA expression of \( \alpha_{1} \) and \( \beta_{1} \)-subunits of ENaC were the same in AEC I and AEC II. However, the expression of \( \gamma \)-subunit of ENaC was five times higher in AEC I than that in AEC II (Fig. 3A). Recently, four subtypes of \( K^{+} \) channels, \( K_{ATP}, \maxi-K_{Ca}, IK_{Ca}, \) and \( KvLQT_1 \), have been identified in alveolar epithelial cells, which belong to three \( K^{+} \) channel classes: inward rectifying \( K^{+} \) channels, voltage-dependent \( K^{+} \) channels, and \( Ca^{2+} \)-activated \( K^{+} \) channels (44, 45). Among four subtypes of \( K^{+} \) channels, the
expression levels of Kir6.1 and SUR2B, which form KATP and maxi-KCa, were very low. No difference was found in IKCa expression between AEC I and AEC II. However, KvTQL1 expression was much higher in AEC II than that in AEC I (Fig. 3B). In addition, we also determined the expression of P2Y2 receptor and Na\(^+\)/H\(^+\)-K\(^+\)/H\(^+\)-2Cl\(^-\)/H\(^+\) cotransporter 1 (NKCC1). There was no difference in P2Y2 receptor expression between AEC I and AEC II. The expression of NKCC1 was higher in AEC II than that in AEC I (Fig. 3C).

We further measured the protein levels of β- and γ-subunits of ENaC by Western blot. Compared with that in AEC II, AEC I had more γ-ENaC protein (Fig. 3D), which is consistent with the result of real-time PCR. Although β-ENaC mRNA was not changed, β-ENaC protein in AEC I was increased compared with AEC II, indicating the difference in the posttranscriptional regulation.

**UTP-induced changes in I\(_{sc}\) were opposite in AEC I and AEC II monolayers.** Subsequently, we examined the effects of UTP on the I\(_{sc}\) in the two types of cell monolayers, AEC I and AEC II. Basolateral application of UTP did not induce any changes on I\(_{sc}\) across AEC I and AEC II monolayers. The K\(_{0.5}\) of amiloride are 1,109 nM and 633 nM in AEC I and AEC II, respectively. N = 5–7 monolayers from 3–4 independent cell preparations.
dramatic and sudden drop in $I_{sc}$, followed by a rapid recovery and then a slow progressive decrease in $I_{sc}$ (Fig. 4B). Given that UTP could be quickly metabolized after it was released under physiological conditions (16, 56), we only focused on the transient effect of UTP in this study. UTP-induced changes in $I_{sc}$ ($\Delta I_{sc}$) were calculated as the peak value of $I_{sc}$ in the presence of UTP minus the basal $I_{sc}$ value in the absence of UTP. The effects of UTP on $\Delta I_{sc}$ were dose dependent in both AEC II and AEC I. The maximal response of UTP occurred at 10 $\mu$M in AEC II. Thus, the concentration of 10 $\mu$M UTP was used in subsequent experiments.

ENaC contributes to the UTP-induced $\Delta I_{sc}$ in AEC I but not in AEC II. Then, we investigated which ions and ion channels contribute to the UTP-induced $\Delta I_{sc}$. First, we tested the involvement of Na$^+$ and Na$^+$ channels in this process. Bathing both sides of cell monolayers with Na$^+$-free Ringer solution decreased the UTP-induced $\Delta I_{sc}$ in AEC II, whereas this almost completely abolished it in AEC I (Fig. 5). Pretreatment of cell monolayers from the basolateral side with 200 $\mu$M ouabain also inhibited the UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II. Application of 20 $\mu$M amiloride to the apical side of cell monolayers almost completely abolished the UTP-induced $\Delta I_{sc}$ in AEC I, whereas this had no effect on the UTP-induced $\Delta I_{sc}$ in AEC II; even the concentration of amiloride was increased to 1 mM (Fig. 5A). Apical application of pimozide (50 $\mu$M) also had no effect on UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II. A combination of amiloride and pimozide did not affect UTP-induced changes in $I_{sc}$ in AEC II. These results indicate that amiloride-sensitive ENaC contributes to the UTP-induced $\Delta I_{sc}$ in AEC I but not in AEC II.
Previous studies have shown that high oxygen tension greatly increased the expression of highly selective cation channels (HSC) (27). Type II cells cultured at a liquid-liquid interface predominantly express nonselective cation channels (NSC). However, the HSC was still the primary channel when type II cells cultured in low oxygen concentration (5%) were exposed to a high oxygen concentration (95%), even for only 2 h. Our AEC II were cultured at an air-liquid interface, and AEC I were cultured at a liquid-liquid interface, which corresponded to high and low oxygen tension, respectively. To address whether the cell culture model affects the ratio of HSC and NSC in AEC II and AEC I, and, in turn, affects the UTP-induced $I_{sc}$ change, the AEC I derived from the type II cells for 4 days at a liquid-liquid interface were exposed to air from several hours to 1 day. Our results showed that the air exposure did not influence UTP-induced change in $I_{sc}$ (data not shown).

CaCC contributes to the UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II. We next examined the contribution of Cl$^{-}$ channels to the UTP-induced $\Delta I_{sc}$. Bathing both sides of cell monolayers with Cl$^{-}$-free Ringer solution blocked the UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II (Fig. 6, A and B). This effect was more profound in AEC I than AEC II. To test which types of Cl$^{-}$ channels mediate the UTP-induced $\Delta I_{sc}$, we pretreated cell monolayers at the apical side with various Cl$^{-}$ channel blockers and tested their effects on the UTP-induced $\Delta I_{sc}$. These blockers included 50 mM NPPB for nonspecific Cl$^{-}$ channels, 200 mM DIDS for CaCC, and 50 mM CFTRinh-172 for CFTR. Given that amiloride completely inhibited UTP-induced $\Delta I_{sc}$ in AEC I, all the effects of Cl$^{-}$ channel antagonists were done in the absence of amiloride. Under these conditions, NPPB, DIDS, and CFTRinh-172 decreased basal $I_{sc}$ in AEC I by 11±2%, 25±5%, and 18±2%, and reduced basal $I_{sc}$ in AEC II by 21±8%, 24±1%, and 21±3% (Table 2). NPPB and DIDS partially abolished the UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II (Fig. 6, A and B). CFTRinh-172 reduced the UTP-induced $\Delta I_{sc}$ in AEC I, but not in AEC II. These data suggest that CaCC is involved in UTP-induced $\Delta I_{sc}$ in AEC II, whereas CaCC and CFTR participate in the UTP effect in AEC I.

K$^{+}$ channels contribute to the UTP-induced $\Delta I_{sc}$ in AEC II but not in AEC I. To test the involvement of K$^{+}$ channels in UTP-induced $\Delta I_{sc}$, 5 mM BaCl$_2$, a general blocker of K$^{+}$ channels, was applied to the basolateral side of the monolayer, and 5 mM mannitol was added into the apical bath solution to prevent the formation of an osmotic gradient. BaCl$_2$ com-

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**Fig. 4.** Effects of UTP on short-circuit current ($I_{sc}$) across AEC I and AEC II monolayers. $I_{sc}$ was measured on AEC II (A) and AEC I (B) monolayers using an Ussing chamber. UTP was applied at the apical side. *Left:* typical tracing curves of $I_{sc}$ induced by 10 μM UTP. *Right:* dose dependence. UTP-induced changes in $I_{sc}$ ($\Delta I_{sc}$) were the differences between the peak value of $I_{sc}$ in the presence of UTP and the basal $I_{sc}$ value in the absence of UTP. Because UTP decreased basal $I_{sc}$ in AEC I, the $\Delta I_{sc}$ was presented as negative values. Data shown are means ± SE ($n$ = 5–6 monolayers from 3–4 independent cell preparations).
trimazole, an inhibitor of IKCa, decreased basal $I_{sc}$ in AEC II, and that K$^+$ channels did not contribute to the UTP-induced $\Delta I_{sc}$ in AEC I.

The signaling pathway for UTP-induced $\Delta I_{sc}$. Because different ion channels are involved in the UTP regulation of $I_{sc}$ in AEC I and AEC II, we wondered whether the signaling transduction pathways mediated by UTP are different in AEC I and AEC II. UTP increases Cl$^-$ secretion in airway epithelial cells by stimulating the P2Y$_2$ receptor, activating phospholipase C (PLC), and increasing intracellular Ca$^{2+}$ concentration via the release of Ca$^{2+}$ from the endoplasmic reticulum (ER) (35). We therefore examined the effects of blocking this pathway on UTP-induced $\Delta I_{sc}$ in AEC I and AEC II (Fig. 8).

Luminal addition of 200 μM suramin, a blocker of the P2Y$_2$ receptor, had no effects on basal $I_{sc}$ but inhibited UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II. Inhibition of PLC by U-73122 or the ER Ca$^{2+}$ pump by thapsigargin resulted in a reduction of basal $I_{sc}$ (97 ± 5% and 25 ± 4% in AEC I: 62 ± 10% and 17 ± 11% in AEC II) (Table 2). UTP-induced $\Delta I_{sc}$ was also dramatically decreased by U-73122.

Complete inhibition of UTP-induced $\Delta I_{sc}$ in AEC II but had no effects on AEC I (Fig. 7). As we mentioned above, there are four subtypes of K$^+$ channels that have been identified in alveolar epithelial cells. Thus, we further investigated which subtypes of K$^+$ channels mediate the effects of UTP in AEC II. Glibenclamide, an inhibitor of K$_{ATP}$, or iberiotoxin, an inhibitor of maxi-K$_{Ca}$, had no obvious effects on UTP-induced $\Delta I_{sc}$ when applied from the basolateral side of the AEC II monolayers (Fig. 7A), which is consistent with very low expression of Kir6.1 and SUR2B in AEC II. However, basolateral treatment with clofilium, an inhibitor of KvLQT$_1$, or clotrimazole, an inhibitor of IK$_{Ca}$, decreased basal $I_{sc}$ by 37 ± 1% and 23 ± 2%, respectively (Table 2), and also decreased UTP-induced $\Delta I_{sc}$ (Fig. 7A). Clotrimazole completely inhibited the UTP-induced $\Delta I_{sc}$. These data suggest that IK$_{Ca}$ and KvLQT$_1$ are involved in UTP-induced $\Delta I_{sc}$ in AEC II and that K$^+$ channels did not contribute to the UTP-induced $\Delta I_{sc}$ in AEC I.

Fig. 5. The involvement of Na$^+$ channels in UTP-induced $\Delta I_{sc}$. For ion substitution experiments, $I_{sc}$ was recorded across AEC II (A) and AEC I (B) monolayers, which were bathed in Na$^+$-free solution at both sides or in normal Ringer solution (control). For the inhibitor studies, the cell monolayers were pretreated with apical amiloride (20 μM or 1 mM), pimozide (50 μM), or ouabain (200 μM) for 3–5 min before addition of UTP (10 μM). Pimozide (50 μM) was applied with or without 20 μM amiloride. The control $\Delta I_{sc}$ was recorded in normal bath solution without any treatments. Data shown are means ± SE (n = 5–6 monolayers from 3–4 independent cell preparations). $^*P < 0.05$ vs. control, $^{**}P < 0.01$ vs. control, $^{***}P < 0.001$ vs. control.

Fig. 6. Effects of Cl$^-$-free solution and Cl$^-$ channel blockers on UTP-induced $\Delta I_{sc}$. In the ion replacement experiment, the UTP-induced $\Delta I_{sc}$ was recorded under conditions where AEC II (A) and AEC I (B) cell monolayers were bathed at both sides with Cl$^-$-free solution, in which Cl$^-$ was replaced by equimolar gluconate$. To detect the effects of Cl$^-$ channel blockers, the monolayers were pretreated with NPPB (50 μM), DIDS (200 μM), or CFTRinh-172 (200 μM), or CFTRinh-172 (50 μM) or CFTRinh-172 (50 μM) on the luminal side for 3–5 min before the addition of UTP (10 μM). The control $\Delta I_{sc}$ was recorded in normal bath solution without any treatments. Data shown are means ± SE (n = 5–6 monolayers from 3–4 independent cell preparations). $^*P < 0.05$ vs. control, $^{**}P < 0.01$ vs. control, $^{***}P < 0.001$ vs. control.
and thapsigargin in both AEC I and AEC II (Fig. 8). A decline of intracellular Ca$^{2+}$ using BAPTA-AM, an intracellular Ca$^{2+}$ chelator, decreased basal $I_{sc}$ as well as UTP-induced $\Delta I_{sc}$ in both AEC I and AEC II. The basal $I_{sc}$ was reduced 40 ± 7% and 12 ± 7% in AEC I and AEC II, respectively, by BAPTA-AM. These results suggest that UTP-mediated signaling transduction pathways that regulate $I_{sc}$ are the same in AEC I and AEC II.

**UTP increased Cl$^-$ secretion in AEC II and Cl$^-$ absorption in AEC I.** UTP induced opposite changes on $I_{sc}$ in AEC I and AEC II, which both involved the apical Cl$^-$ channels, CaCC. However, the inhibition of the Cl$^-$ channels, CaCC, similarly decreased UTP-induced $\Delta I_{sc}$. We hypothesize that the difference in UTP-induced changes on $I_{sc}$ between AEC I and AEC II is due to the opposite Cl$^-$ flux caused by UTP. To test this hypothesis, we examined the effects of UTP on the unidirectional Cl$^-$ transport across AEC I and AEC II monolayers. In the absence of UTP, there was no significant difference of Cl$^-$ flux between basolateral to apical and apical to basolateral in both AEC I and AEC II (Fig. 9). UTP (10 $\mu$M) markedly increased basolateral-to-apical Cl$^-$ transport but had no effects on apical-to-basolateral Cl$^-$ transport in AEC II, which resulted in the increase of net basolateral-to-apical Cl$^-$ flux from 1.33 ± 1.01 to 6.65 ± 0.84 nmol/cm$^2$/h (Fig. 9A). In contrast, treatment of AEC I monolayer with 10 $\mu$M UTP greatly increased apical-to-basolateral Cl$^-$ transport but had no significant effects on basolateral-to-apical Cl$^-$ transport. The net value of apical-to-basolateral Cl$^-$ transport was augmented from 1.65 ± 0.85 to 8.32 ± 2.69 nmol/cm$^2$/h (Fig. 9B). These results suggest that, in the presence of UTP, the direction of net Cl$^-$ flux in AEC II and AEC I is opposite. UTP increased net Cl$^-$ secretion in AEC II but increased Cl$^-$ absorption in AEC I.

**Effects of dexamethasone and TGF-$\beta$ on UTP-induced $\Delta I_{sc}$ in AEC I and AEC II monolayers.** It is known that dexamethasone increases expression and activities of amiloride-sensitive ENaC (27). Since amiloride-sensitive ENaC is involved in UTP-induced $\Delta I_{sc}$ in AEC I, we further tested the effects of dexamethasone on UTP-induced $\Delta I_{sc}$. The AEC I and AEC II monolayers were cultured in the presence of 1 $\mu$M dexamethasone from day 0 (D0). The results showed that dexamethasone increased the basal $I_{sc}$ from 7.33 ± 0.32 to 11.89 ± 1.03 $\mu$A/cm$^2$ ($n = 9$) in AEC I and from 5.12 ± 0.19 to 9.62 ± 1.03 $\mu$A/cm$^2$ ($n = 8$) in AEC II, respectively. Dexamethasone did not affect UTP-induced $\Delta I_{sc}$ in AEC I (Fig. 10A). However, UTP induced a biphasic $I_{sc}$ change in AEC II monolayers when cultured in the presence of dexamethasone. UTP induced a transient fall in $I_{sc}$, followed by a rise to a peak value, and then gradually declining to below the baseline (Fig. 10A). These results suggest that increasing the expression of ENaC in AEC II might enhance the involvement of ENaC in UTP-induced $\Delta I_{sc}$.

Our previous studies have shown that TGF-$\beta$ regulates the transdifferentiation of AEC II to AEC I though the Smad pathway (3). Thus, we further explored whether UTP-induced $\Delta I_{sc}$ in AEC I and AEC II could be influenced by TGF-$\beta$. TGF-$\beta$ (10 ng/ml) was added to culture medium from D0 in both AEC I and AEC II. TGF-$\beta$ decreased the basal $I_{sc}$ to 3.2 ± 0.23 $\mu$A/cm$^2$ ($n = 9$) and 4.33 ± 0.29 $\mu$A/cm$^2$ ($n = 8$) in the AEC II and AEC I monolayer, respectively. Compared with the control, UTP induced a similar increase in $I_{sc}$ in AEC II monolayers. However, UTP induced a biphasic $I_{sc}$ change in AEC I monolayers (Fig. 10B). The $I_{sc}$ quickly fell to a peak value, followed by a rise to above the baseline.

**Effect of CFTR inhibitor on terbutaline-stimulated $I_{sc}$.** $\beta$-adrenergic agonist stimulates Cl$^-$ flux in the alveolar epithelial monolayer. We examined the effects of terbutaline on $I_{sc}$ in AEC I and AEC II and their sensitivities to CFTR inhibitor. Basal application of terbutaline (10 $\mu$M) induced an increase in $I_{sc}$ in AEC II, whereas a decrease in $I_{sc}$ in AEC I (Fig. 11). Terbutaline-induced changes were reversed by apical treatment of 50 $\mu$M CFTRinh-172, the inhibitor of CFTR. This is consistent with opposite directions of Cl$^-$ flux between AEC I and AEC II. Using a similar air-lipid model, Jiang et al. (29) observed that terbutaline induced a decrease of $I_{sc}$. However, they cultured type II cells for 9–10 days, whereas we only cultured for 2–3 days.

**DISCUSSION.** As an auto/paracrine molecule, UTP regulates ion transport in nearly all secretory and absorptive epithelia via the activation of P2Y receptors. Our present study indicated that UTP increased $I_{sc}$ in AEC II, whereas UTP decreased it in AEC I via different mechanisms. In AEC II, the apical Cl$^-$ channel, CaCC, and the basolateral K$^+$ channel, IK$_{Ca}$, were the main

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**Table 2. Effect of blockers of ion channels and transporters on basal $I_{sc}$ of AEC I and AEC II monolayers**

<table>
<thead>
<tr>
<th>Agents</th>
<th>$I_{sc}$ (no drug)</th>
<th>$I_{sc}$ (drug)</th>
<th>Inhibition (%)</th>
<th>$P$ value</th>
<th>$I_{sc}$ (no drug)</th>
<th>$I_{sc}$ (drug)</th>
<th>Inhibition (%)</th>
<th>$P$ value</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPPB</td>
<td>4.85 ± 0.51</td>
<td>3.20 ± 0.23</td>
<td>21 ± 8</td>
<td>&lt;0.05</td>
<td>6.16 ± 0.5</td>
<td>5.52 ± 0.48</td>
<td>11 ± 2</td>
<td>&lt;0.001</td>
<td>6</td>
</tr>
<tr>
<td>DIDS</td>
<td>5.95 ± 0.61</td>
<td>4.50 ± 0.45</td>
<td>24 ± 7</td>
<td>&lt;0.01</td>
<td>7.38 ± 0.99</td>
<td>5.65 ± 0.97</td>
<td>25 ± 5</td>
<td>&lt;0.01</td>
<td>6</td>
</tr>
<tr>
<td>CFTRinh-172</td>
<td>4.74 ± 0.46</td>
<td>3.80 ± 0.51</td>
<td>21 ± 3</td>
<td>&lt;0.01</td>
<td>7.18 ± 0.22</td>
<td>5.87 ± 0.25</td>
<td>18 ± 2</td>
<td>&lt;0.001</td>
<td>6</td>
</tr>
<tr>
<td>Gilbenclamide</td>
<td>4.41 ± 0.19</td>
<td>3.77 ± 0.16</td>
<td>15 ± 2</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>Clofilium</td>
<td>5.32 ± 0.62</td>
<td>3.31 ± 0.33</td>
<td>37 ± 1</td>
<td>&lt;0.01</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>5.08 ± 0.83</td>
<td>3.97 ± 0.69</td>
<td>23 ± 2</td>
<td>&lt;0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iberiotoxin</td>
<td>4.92 ± 0.45</td>
<td>5.0 ± 0.41</td>
<td>2 ± 1</td>
<td>NS</td>
<td>7.06 ± 0.49</td>
<td>4.12 ± 0.42</td>
<td>40 ± 7</td>
<td>&lt;0.05</td>
<td>6</td>
</tr>
<tr>
<td>BAPTA-AM</td>
<td>5.37 ± 0.70</td>
<td>4.73 ± 0.71</td>
<td>12 ± 7</td>
<td>&lt;0.05</td>
<td>8.25 ± 0.52</td>
<td>6.21 ± 0.56</td>
<td>25 ± 4</td>
<td>&lt;0.05</td>
<td>5</td>
</tr>
<tr>
<td>Thapsigargin</td>
<td>5.25 ± 0.85</td>
<td>3.66 ± 0.98</td>
<td>17 ± 11</td>
<td>&lt;0.05</td>
<td>6.77 ± 0.48</td>
<td>0.20 ± 0.42</td>
<td>97 ± 5</td>
<td>&lt;0.05</td>
<td>6</td>
</tr>
<tr>
<td>U-73122</td>
<td>5.46 ± 0.64</td>
<td>1.79 ± 0.26</td>
<td>62 ± 10</td>
<td>&lt;0.05</td>
<td>—</td>
<td>—</td>
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</table>

The basal $I_{sc}$ were measured in the absence (no drug) or presence (drug) of ion channel blockers. The $I_{sc}$ inhibition (%) was also indicated. The number of cell monolayers measured from at least 3 independent cell preparations is indicated by $n$. $P$ value was obtained by comparing $I_{sc}$ before and after application of drugs. NS, no significance.
contributors to the UTP effect. In AEC I, the apical Na$^+$ channel, ENaC, and the CaCC determined the UTP-induced $I_{sc}$.

These results suggest that AEC I and AEC II play different roles in the UTP regulation of ion channels.

Type I cells have been isolated successfully (9, 33). Patch clamp has been performed on isolated type I cells, and several ion channels including ENaC, CFTR, and K$^+$ channels have been detected (33). However, electrophysiological characteristics of ion transport on cell monolayers formed from isolated type I cells have not been reported yet, probably because of the difficulty to form a monolayer on permeable supports that are suitable for Ussing chamber measurement. It is well known that type II cells undergo transdifferentiation to type I cells when cultured on permeable membrane under liquid-liquid culture conditions. Although they are not ideal, the transdifferentiated cells are used as an in vitro model for type I cells. On the other hand, type II cells cultured under air-liquid conditions are usually utilized to maintain the phenotype of type II cells. Both culture models have been used for ion transport in literature (15, 20, 29). In the current study, we used similar models to represent AEC I and AEC II and confirmed specific cell marker expression in each model.

Our electrophysiological studies indicated the PD and basal $I_{sc}$ across AEC I monolayers are higher than that of AEC II monolayers, suggesting that ion transport is different between AEC I and AEC II. Ion substitution experiments demonstrated that both Na$^+$ and Cl$^-$ contributed to basal $I_{sc}$. Although previous studies have reported the absence of Cl$^-$ flux in basal condition (20), our current studies revealed that Cl$^-$ blockers...
shown are means affected by culture conditions in vitro, may determine basal but not in AEC I. Thus, cell phenotypes, which are CFTRinh-172 only inhibited basal the presence of amiloride, apical application of NPPB and side in the absence (control) or presence (UTP) of 10 due to culture conditions. activities between our study and previous studies may be slightly inhibited basal (≈20%, Table 2). Furthermore, in the presence of amiloride, apical application of NPPB and CFTR inh-172 only inhibited basal in AEC II, but not in AEC I, implying that there is Cl– flux from CFTR in AEC II but not in AEC I. Thus, cell phenotypes, which are affected by culture conditions in vitro, may determine basal Cl– channel activities. The differences in basal Cl– channel activities between our study and previous studies may be due to culture conditions.

Patch-clamp studies revealed that both type I and type II cells had HSC and NSC (33). HSC was very sensitive to amiloride (K0.5 < 50 nM), consistent with the properties of the ENaC composed of α-, β-, and γ-subunits. NSC was only inhibited by high concentrations of amiloride (K0.5 ≈ 1 mM), consistent with the ENaC formed from the α-subunit alone. The channel density of HSC was more than 10 times that of NSC in both freshly isolated type I and type II cells (33). Jain et al. showed that type II cells mainly expressed NSC when they were cultured on a plastic plate or glass in the absence of dexamethasone. However, culturing type II cells on permeable filter with its apical side exposed to air was sufficient to increase HSC expression (27). In our study, the application of amiloride to the apical sides of both AEC I and AEC II monolayers dramatically decreases their basal Isc (58% and 74%) with K0.5 of 1.109 nM and 633 nM, respectively. These results indicate that NSC is not highly expressed in both types of cells under our culture conditions, consistent with freshly isolated type I and type II cells.

There is a difference in oxygen tension on the apical surface of the monolayers between air-liquid (AEC II) and liquid-liquid (AEC I) models. AEC II was exposed to a higher oxygen concentration compared with AEC I. Previous studies demonstrated that a high oxygen concentration (95%) dramatically increased the expression of HSC (27), whereas hypoxia reduced the expression of CFTR (1, 23) and ENaC subunits (especially β and γ) in cultured alveolar epithelial cells or other epithelial cells (28, 58). The lack of the effects of CFTR inh-172 on the amiloride-insensitive Isc in AEC I (liquid-liquid) may be due to its hypoxic conditions. However, AEC II should have more amiloride-sensitive ENaC than AEC I. In contrast, our current data indicated that AEC II actually had less amiloride-sensitive ENaC (58%) than AEC I (74%). The controversy may be due to the different culture conditions. Compared with previous studies of high oxygen tension (95%), our AEC II was exposed to normal oxygenation (21%), and the liquid layer above the surface of AEC I was thin (0.3 ml) and may not be enough to create severe hypoxic conditions.

In the airway epithelium, UTP stimulates an alternative Cl– channel, CaCC, and enhances Cl– secretion (48). CaCCs are anion-sensitive channels activated by a rise in cytosolic Ca2+ (17). Although the biophysical characteristics of CaCCs are well understood and CaCCs have been shown to be involved in many different physiological processes such as excitability of neurons and muscle cells and transepithelial transport, the molecular identity of CaCCs is still unknown. Pharmacologically, CaCCs are sensitive to DIDS and NPPB (17, 63). In our study, UTP increased Isc in AEC II that was markedly inhibited by pretreatment with DIDS and NPPB, suggesting that UTP induces Cl– secretion in AEC II by activating CaCC. Although it mediates Cl– transport on the apical side of alveolar epithelia (6, 33), CFTR does not contribute to the regulation of Isc by UTP in AEC II since the CFTR inhibitor, CFTR inh-172, had no effects on UTP-induced ΔIsc in AEC II. Application of DIDS and NPPB also markedly inhibited UTP-induced ΔIsc in AEC I. Since UTP induced a decrease of Isc in AEC I, UTP promotes Cl– absorption rather than secretion in AEC I. This was supported by the fact that UTP increased net Cl– absorption in AEC I and Cl– secretion in AEC II as determined by radioisotope measurements.
In utero, Cl\(^{-}\) was shown to actively secrete into alveoli. This process promotes fluid secretion and in turn stimulates lung growth. Just before birth, alveolar fluid is cleared (50). In adult lung, Cl\(^{-}\) is absorbed through CFTR in both basal and stimulated conditions (19). \(\beta\)-adrenergic agonists activate apical Cl\(^{-}\) channels, resulting in Cl\(^{-}\) influx from the apical side in cultured adult alveolar type II cells (29, 30). However, our recent studies have demonstrated that GABAA receptors mediate Cl\(^{-}\) secretion in freshly isolated adult alveolar type II cells (31). In the airway epithelium, UTP stimulates Cl\(^{-}\) secretion via activation of CaCCs, and for this reason, UTP is considered as a promising compound for potential CF therapy (2, 36). Thus, Cl\(^{-}\) flux direction is determined by developmental stages and cell types.

Cl\(^{-}\) flux direction through Cl\(^{-}\) channels depends on the electrochemical gradient across the epithelial membrane. One of the possible reasons for the opposite Cl\(^{-}\) transport in AEC I and AEC II is their difference in PD. Our results indicated that the values of PD were higher in AEC I than those in AEC II. This would lead to the difference in the electrical gradient for Cl\(^{-}\) transport. ENaC is a main ion channel that contributes to PD, which is composed of three subunits, \(\alpha\), \(\beta\), and \(\gamma\) (64). Both AEC I and AEC II contain all three subunits of ENaC (4, 34), but a recent study has shown that all ENaC subunit transcripts are higher in AEC I than in AEC II cells (33). Our current study revealed that there was no significant difference in \(\alpha\)-ENaC and \(\beta\)-ENaC mRNA between our AEC I and AEC II preparations. However, expression levels of \(\gamma\)-ENaC mRNA and protein as well as \(\beta\)-ENaC protein in AEC I were higher than that of AEC II. The results suggest that \(\beta\)-ENaC and \(\gamma\)-ENaC are probably regulated at the transcriptional and posttranscriptional levels, respectively. This is also consistent with the fact that the amiloride-sensitive \(I_w\) was higher in AEC I than that in AEC II. Thus, the alterations in the ENaC expression levels may lead to the difference in PD, and, in turn, in electrical gradient for Cl\(^{-}\) transport between AEC I and AEC II.

Another possible reason for the opposite Cl\(^{-}\) transport may be due to the difference of intracellular Cl\(^{-}\) concentrations in AEC I and AEC II, which are controlled by basolateral Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter 1 (NKCC1). Cl\(^{-}\) is transported from the basolateral side into epithelial cells by NKCC1. The inhibition of NKCC1 with bumetanide decreases Cl\(^{-}\) secretion in secretory epithelia (24). Our results indicated that the NKCC1 mRNA expression was higher in AEC II than in AEC I. This is consistent with a previous report that the NKCC1 activity was dramatically decreased in cultured primary AEC II, which was presumably trans-
indicating that the activation of CaCC and thus Cl\(^{-}\) involvement mechanism is still not clear. There is evidence of sorption in AEC I, which is consistent with the result from suggesting that UTP inhibited ENaC activity and Na\(^{+}\) contributed to the UTP-induced decrease of I\(_{sc}\). A low expression of NKCC1 differentiated into AEC I (10). A low expression of NKCC1 in AEC I may lead to a lower intracellular Cl\(^{-}\) concentration in AEC I compared with that in AEC II, which, in turn, generates a lower chemical gradient for Cl\(^{-}\} secretion from the luminal side.

Our results showed that amiloride-sensitive ENaC contributed to the UTP-induced decrease of I\(_{sc}\) in AEC I, suggesting that UTP inhibited ENaC activity and Na\(^{+}\} absorption in AEC I, which is consistent with the result from the airway and other epithelial cells (13, 26, 48). The involved mechanism is still not clear. There is evidence indicating that the activation of CaCC and thus Cl\(^{-}\} influx was crucial for the inhibition of ENaC by UTP (38, 48). This effect may be exerted by an unidentified Cl\(^{-}\} sensitive protein that is activated by the increased intracellular Cl\(^{-}\} (38). However, other studies have demonstrated that the inhibition of ENaC activity during stimulation of P2Y receptors with ATP is mediated by the hydrolysis of PI(2,3)P2, and not an increase in intracellular Ca\(^{2+}\} (37, 39).

In contrast to AEC I, ENaC did not contribute to the UTP regulation of ion transport in AEC II since amiloride had no effects on the UTP-induced change in I\(_{sc}\}; even the concentration of amiloride was increased to 1 mM. \(\alpha\)-ENaC subunit alone can form functional channels, and \(\beta\)- and \(\gamma\)-ENaC subunits require coassembly with the \(\alpha\)-ENaC subunit (8, 52). Thus, the \(\alpha\)-ENaC subunit is the crucial one to form the Na\(^{+}\} channel itself, and \(\beta\)- and \(\gamma\)-ENaC subunits are regulatory subunits. Recently, Elias et al. (18) showed that \(\beta\)- and \(\gamma\)-ENaC subunits, but not the \(\alpha\)-ENaC subunit, play a crucial role in the regulation of net fluid absorption by edema fluid in murine distal lung epithelia. Since AEC I contained more \(\gamma\)-ENaC transcripts than AEC II, it is possible that the \(\gamma\)-ENaC subunit provides the regulatory sites for UTP. Thus, the different contributions of ENaC to the regulation of I\(_{sc}\} by UTP in AEC I and AEC II may be due to their subunit expression levels.

Epithelial K\(^{+}\} channels play an essential role in transepithelial ion and water transport (55). Recently, several K\(^{+}\} channel mRNA and/or proteins were detected in type II cells including Kir6.1 and SUR2B (to form K\(_{ATP}\} channels), KvLQT1, IKCa, and maxi-KCa (44, 45). In the current study, we detected a similar amount of the IKCa mRNA in AEC I and AEC II. The KvTQL1 mRNA level in AEC II was higher than that in AEC I. However, the mRNA expression of maxi-KCa, Kir6.1, and SUR2B was very low in both cell types, which was not consistent with the previous study. The discrepancy may be due to different cell culture conditions.

Our functional experiments indicated that basolateral K\(^{+}\} channels were involved in the UTP-induced ΔI\(_{sc}\} in AEC II. Among four K\(^{+}\} channels examined, IKCa was the main channel contributing to this effect. However, KvLQT1 appears to have effects, although to a lesser content. A previous study has demonstrated that, in secretory epithelia, the parallel activation of basolateral K\(^{+}\} channels with apical Cl\(^{-}\} channels is required to generate a driving force for Cl\(^{-}\} exit across the apical membrane (55). The inhibition of basolateral IKCa abolished the UTP-induced increase in I\(_{sc}\}, suggesting that the activation of apical Cl\(^{-}\} secretion via CaCC in AEC II may also require the parallel activation of basolateral IKCa. A similar result was observed in human airway epithelia (47). Both CaCC and IKCa are activated by an increase of intracellular Ca\(^{2+}\}. Thus, an increase in I\(_{sc}\} induced by UTP in AEC II was mediated by both apical Cl\(^{-}\} channels and basolateral K\(^{+}\} channels. This may explain why Cl\(^{-}\} channel blockers only partly blocked the effects of UTP in AEC II.

Basolateral treatment of AEC I monolayers with BaCl\(_{2}\}, a general K\(^{+}\} blocker, had little effect on basal I\(_{sc}\} and UTP-induced ΔI\(_{sc}\}, suggesting that K\(^{+}\} channels did not contribute to the ion transport in AEC I. There are several possibilities for the differential contribution of K\(^{+}\} channels to the UTP effects in AEC I and AEC II: 1) although the mRNA levels of IKCa are the same in AEC I and AEC II, IKCa protein levels or their posttranslational regulations may be different in the two types of cells; 2) the effects observed may be due to the differential expression of the KvLQT1; and 3) the difference in intracellular Cl\(^{-}\} concentration in AEC I and AEC II may lead to the differential activation of K\(^{+}\} channels.

The opposite effects induced by UTP on I\(_{sc}\} in AEC I and AEC II are not due to the changes of P2Y\(_{2}\} receptor signaling. This is supported by the following observations: 1) P2Y\(_{2}\} receptor expression levels are the same in AEC I and AEC II; 2) the blockage of the P2Y\(_{2}\} receptor with suramine similarly inhibited the UTP-induced ΔI\(_{sc}\} in AEC I and AEC II; 3) the
UTP-induced $\Delta l_o$ in both AEC I and AEC II was greatly blocked by BAPTA-AM, an intracellular Ca$^{2+}$ chelator.

Based on the current studies, we propose a model for differential regulation of ion transport by UTP in alveolar epithelial cells (Fig. 12). UTP activates the P2Y2 receptor and increases intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) via phospholipase C (PLC). In AEC II (A), the increased Ca$^{2+}$ stimulates the basolateral intermediate conductance Ca$^{2+}$-activated K$^+$ channel (IKCa) and the apical Ca$^{2+}$-activated Cl$^-$ channel (CaCC). This leads to an increase in Cl$^-$ secretion from the apical side and K$^+$ secretion from the basolateral side. In AEC I (B), a rise of Ca$^{2+}$ inhibits the apical ENaC and activates CaCC, leading to the influx of Cl$^-$ because of its low intracellular Cl$^-$ concentration.

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