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RESEARCH ARTICLE

Concurrent absorption and secretion of airway surface liquids and bicarbonate secretion in human bronchioles

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Shamsuddin AK, Quinton PM. Concurrent absorption and secretion of airway surface liquids and bicarbonate secretion in human bronchioles. *Am J Physiol Lung Cell Mol Physiol* 316: L953–L960, 2019. First published March 6, 2019; doi:10.1152/ajplung.00545.2018.—Although small airways account for the largest fraction of the total conducting airway surfaces, the epithelial fluid and electrolyte transport in small, native airway epithelia has not been well characterized. Investigations have been limited, no doubt, by the complex tissue architecture as well as by its inaccessibility, small dimensions, and lack of applicable assays, especially in human tissues. To better understand how the critically thin layer of airway surface liquid (ASL) is maintained, we applied a "capillary"-Ussing chamber (area ≈1 mm^2) to measure ion transport properties of bronchioles with diameters of ~2 mm isolated from resected specimens of excised human lungs. We found that the small human airway, constitutively and concurrently, secretes and absorbs fluid as observed in porcine small airways (50). We found that the human bronchiolar epithelium is also highly anion selective and constitutively secretes bicarbonate (HCO_3^-), which can be enhanced pharmacologically by cAMP as well as Ca^{2+}-mediated agonists. Concurrent secretion and absorption of surface liquid along with HCO_3^- secretion help explain how the delicate volume of the fluid lining the human small airway is physiologically buffered and maintained in a steady state that avoids desiccating or flooding the small airway with ASL.

airway surface liquid; ASL; CFTR; Cl^- transport; ENaC; transport

INTRODUCTION

Even though distal small airways are a major site of lung pathogenesis, characterization of the epithelial ion transport functions of the bronchiole has been limited by the complex anatomy and relative inaccessibility of these structures, especially in humans. Small airways comprise the more distal zones of the human bronchial tree with diameters usually less than ~2 mm that account for the largest portion of the total surface area of the conducting structures of the lung. Their ciliated lumens extend approximately from generations 8–9 to 13–15 (59). By definition small airways lack surrounding cartilaginous rings and submucosal glands and therefore, unlike larger bronchi, are collapsible. We observed that freshly dissected adult human small airways of less than ~2 mm in diameter are collapsed with little, if any, cartilage present in the bronchiolar wall.

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To date, we have only a limited understanding of how the airways manage the volume (critical thickness) of airway surface liquid (ASL) so precisely that normally the airway never becomes occluded with excessive ASL nor desiccated from a lack of it. Since it is not possible for an epithelial cell to transport fluid simultaneously in opposite directions, a common concept has held that the mucosal lumen is lined with a uniform epithelium that as a unit regulates the ASL volume/thickness by alternating between fluid absorption and secretion (10, 11, 29).

Since we know of no other example of epithelial cells that can acutely reverse fluid transport directions, we asked whether the mucosal epithelia might actively absorb and secrete electrolytes simultaneously via distinct subsets of luminal epithelial cells, one that continuously adds and another that continuously removes fluid along the airway surface (15, 50). To investigate these fluid transport properties, we applied a unique capillary-Ussing chamber (area ≈1 mm^2) (50) to measure transepithelial electrophysiological properties of small specimens of native, intact, freshly dissected, human small airways.

Since patency of these airways is critically affected by inflammation and mucus accumulation in lung diseases such as cystic fibrosis (27, 40, 48, 53), asthma (9, 57), and chronic obstructive pulmonary disease (9, 52), and since HCO_3^- secretion is required for normal mucus formation (2, 16, 18, 24, 31, 36, 56) as well as for innate defense, neutrophil responses, and bacterial viability (13, 14, 27, 33, 36, 38), we examined human small airways for properties of HCO_3^- secretion (49).

We find that the fluid and electrolyte transport properties of human airways appear to be similar to those observed in porcine small airway, which further supports the hypothesis that separate cells independently secrete and absorb ASL (35, 50). Moreover, human native small airway epithelia also, like porcine lung (49), not only secrete bicarbonate constitutively, but cAMP and Ca^{2+}-mediated agonists significantly stimulate HCO_3^- secretion.

MATERIALS AND METHODS

Tissue procurement. We procured excess freshly resected human lungs from 25 donor subjects at regional hospitals via Life Share (a Donate Life organization) and two lobectomies over the period from 2008 to 2018 that provided specimens sufficiently viable and intact to exhibit spontaneously active transepithelial electrical currents. The Internal Review Board of University of California, San Diego approved the procedures in this study. Immediately after receiving the resected lung tissue, we placed specimens in plastic bags under

crushed ice to cool and preserve tissue viability until used, usually within 2–4 h.

Bronchiole dissection and mounting. We cut small blocks of ~1–3 cm^3 from the peripheral lung parenchyma, usually from the costal diaphragmatic ridge of the lower lobes of whole lungs that were then placed in a chilled (<10°C) bath of NaCl Ringer solution for microdissection of the bronchioles. Under a dissecting microscope, we identified the exposed transected lumens of small airways (~2 mm ø) on the surfaces of sliced blocks of tissue and isolated 2- to 4-mm lengths of the identified airway from surrounding parenchyma with sharpened tweezers and iridectomy scissors. We slit the lumen longitudinally to expose about ~4–6 mm^2 of luminal surface, which was then mounted with 00 insect pins on a "trampoline" made of an open-weave nylon mesh glued over the end of a fabricated plastic tube (inner diameter: 3 mm; outer diameter: 7 mm), the perfused interior of which constituted the serosal fluid compartment of the capillary-Ussing chamber. We used a V-track concentric pipette carrier designed originally for renal microtubule perfusion (12) to gradually advanced the end of a carefully squared, fire polished glass capillary (inside area: 0.66 mm^2) against the luminal surface of the airway epithelial surface that was pinned flat over the supporting trampoline (50). Thus the capillary lumen formed the luminal compartment of the capillary-Ussing chamber for measuring transepithelial electrical properties. In the presence of a 150 mM Cl^- serosal to mucosal gradient, the capillary was gradually advanced to achieve an apparent maximum transepithelial potential (V_t). Preparations that did not spontaneously exhibit a V_t of at least 20-mV lumen negative in the presence of the 150 mM Cl^- transepithelial gradient were discarded. Both the apical and serosal sides of the tissue were perfused as needed with defined solutions at ~37°C.

Electrophysiological measurements. We measured open circuit V_t continuously via free solution bridges of 3 M KCl from Ag-AgCl electrodes that formed rinseable junctions with the stream of perfusates leaving the tissue. Rinsing the "perfusate:KCl bridge" junction after each change of perfusates with fresh KCl solution minimized and stabilized liquid junction potentials. Electrode asymmetries were measured before and after mounting each tissue, which were usually <0.5 mV. All reported values are measured with asymmetries subtracted. Transepithelial conductance (G_t) was calculated from Ohm's law using the deflection in V_t that resulted from constant 1.0-μA current pulses (1.5 s duration) passed across the tissue at 10-s intervals. I_{sc}^{eq} was calculated from G_t and the open circuit V_t using Ohm's law.

All assays of electrogenic HCO_3^- transport were carried out in bilateral 25 mM HCO_3^- plus 125 mM NaGlu Ringer solution in the presence of luminal amiloride to block electrogenic Na^+ absorption and prevent Cl^- secretion (49). We added cAMP- and Ca^{2+}-mediated agonists to the serosal media to stimulate secretion while recording changes in I_{sc}^{eq} as calculated from measured V_t and G_t. Since no significantly transportable anion other than HCO_3^- was present, the changes in V_t, G_t, and I_{sc}^{eq} following stimulation were interpreted as reflecting active HCO_3^- secretory transport. The fact that known HCO_3^- transport inhibitors almost completely blocked these I_{sc}^{eq}s supported this interpretation. Accordingly, when no HCO_3^- was present under these conditions (gluconate substituted for Cl^- bilaterally), inhibitors had no discernible effects (data not shown).

Solutions and drugs. The Ringer solution contained in the following (in mM): 150 Na^+, 4.6 K^+, 1.0 Mg^{2+}, 1.0 Ca^{2+}, 150 Cl^-, 1.0 SO_4^{2+}, 2.5 phosphate, 2.0 acetate, and 10 glucose buffered to pH 7.4 with HCl. To minimize endogenously generated prostaglandins during dissection, indomethacin (1 μM) was present in the dissecting Ringer solution. For anion diffusion studies, 150 mM NaCl was replaced with equimolar Na^+ gluconate or with 75 mM NaCl plus 150 mM mannitol for osmolar balance. For $NaHCO_3^-$ Ringer solution, 25 mM $NaHCO_3^-$ plus 125 Na^+ gluconate was substituted for Cl^- as indicated. All HCO_3^- solutions were adjusted to pH 7.4 by gassing to equilibrium with 95% O_2 plus 5% CO_2.

3-Isobutyl-1-methylxanthine (IBMX; 100 μM), forskolin (Fsk; 10 μM), UTP (100 μM), amiloride (10 μM), niflumic acid (NFA; 100 μM), indomethacin (1 μM), and bumetanide (100 μM) were all obtained from Sigma Chemical (St. Louis, MO) and used at the concentrations indicated in parentheses. The cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor GlyH-101 (50 μM) was a generous gift from Dr. A. Verkman and Dr. R. Bridges. Luminal and basolateral solutions were changed rapidly via manifolds that distributed stores of the above solutions as required.

Statistical analysis. The data are presented as means ± SE if normally distributed, and "*n*" is the number of tissues examined. Box plots show median, upper, and lower quartiles and whiskers show upper and lower extreme data points. Statistical significance was determined on the basis of Student's paired *t*-test with $P < 0.05$ taken as significantly different.

RESULTS

Spontaneous V_t, G_t, and I_{sc}^{eq}. In the presence of bilateral NaCl Ringer solution, the mean values of spontaneous transepithelial potential (V_t), conductance (G_t), and equivalent short circuit current (I_{sc}^{eq}) were −3.3 ± 0.4 mV (lumen negative), 16.5 ± 2.2 mS/cm^2, and 55.2 ± 11.4 μA/cm^2, respectively (means ± SE; $n = 21$; mean airway diameter: 1.9 ± 0.1 mm; Table 1). The highest V_t in bilateral 150 mM NaCl was −6.0 mV. Table 1 compares the spontaneous ion transport parameters of human small airways observed herein with those of pig (50).

Bi-ionic Cl^- diffusion potentials. Luminal substitution of NaGlu for NaCl, that is, replacing the highly permeant Cl^- anion with the impermeant gluconate anion, significantly hyperpolarized V_t by eightfold and decreased G_t by ~50%, demonstrating an inherent, predominant transepithelial Cl^-conductance that appears to be constitutively present (Table 1, Fig. 1). The maximum V_t of human small airways bathed with 150 mM NaCl on the serosa and 150 mM NaGlu on the luminal mucosa was − 45.7 mV with a mean V_t of −26.4 ± 1.4 ($n = 21$). Table 1 compares similar data for porcine (50) and human small airways.

Table 1. *Basic electrophysiological parameters of native small airways of pig and humans: spontaneous V_t, G_t, and I_{sc}^{eq} and bi-ionic V_t, G_t*

Lumen/Bath	Pig*	Human
n	37	21
NaCl/NaCl	−2.1 ± 0.2	−3.3 ± 0.4
V_t, mV	21.2 ± 0.9	16.5 ± 2.2
G_t, mS/cm^2	44.4 ± 3.9	55.2 ± 11.4
I_{sc}^{eq}, μA/cm^2		
V_t, mV	−37.7 ± 1.6	−26.4 ± 1.4
G_t, mS/cm^2	9.4 ± 0.6	10.5 ± 1.4

Values are means ± SE; n = number of measurements in as many tissues with mean diameters of 1.9 ± 0.1 mm (Human) and 1.1 ± 0.2 mm (Pig). The luminal surface of the tissue was perfused with either 150 mM NaCl or 150 mM NaGlu while the serosal surface was bathed continuously in 150 mM NaCl. The open circuit transepithelial potential (V_t) was measured continuously; transepithelial conductance (G_t) was calculated from Ohm's law from the change in V_t during transepithelial constant current pulses (~1 μA); and equivalent short circuit current (I_{sc}^{eq}) was calculated from G_t and spontaneous V_t. *Data from pigs (50).

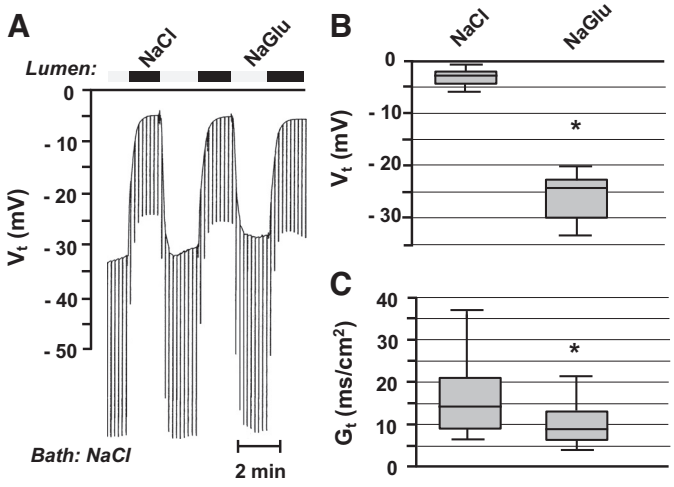

Fig. 1. Bi-ionic Cl^- diffusion potentials in human small airways. *A*: representative electrical trace of transepithelial potential (V_t) with constant current pulses (0.5 μA) on the effect of substituting luminal gluconate, an impermeant anion, for the permeable Cl^- anion on a dissected small airway mounted in a capillary-Ussing chamber. Replacing luminal Cl^- with gluconate hyperpolarized the V_t significantly, indicating a predominant Cl^- conductance that appears to be constitutively active. *B*: box plots showing median, upper, and lower quartiles and whiskers showing upper and lower extreme data points of the results for V_t from similar experiments. When 150 mM NaGlu replaced luminal 150 mM NaCl, V_t hyperpolarized markedly ($P < 0.001$; $n = 21$). *C*: box plots as in *B* for changes in transepithelial conductance (G_t). Substituting gluconate for luminal Cl^- significantly decreased G_t. *$P < 0.001$; $n = 21$.

Inhibition of absorption and secretion. We examined the effects of selectively inhibiting absorption and secretion in human native small airways. Applying amiloride (10 μM) to the luminal surface to block Na^+ conductance and inhibit Na^+ absorption through epithelial sodium channels decreased the spontaneous V_t, G_t, and I_{sc}^{eq} by 35.3, 13.2, and 45.7%, respectively, of the initial spontaneous values in symmetric 150 mM NaCl-Ringer solutions (Fig. 2). Applying bumetanide to the basolateral side of the tissue to inhibit the Na^+-K^+-$2Cl^-$ cotransporter in the basolateral membrane and block Cl^--dependent secretion further inhibited the spontaneous V_t, G_t, and I_{sc}^{eq} by 86.4, 9.6, and 89.1%, respectively (Fig. 2). Thus concurrent inhibition with inhibitors of absorption and secretion essentially abolished the I_{sc}^{eq}.

Opposite effects of cAMP and UTP on V_t. Since CFTR and Ca^{2+}-mediated Cl^- channels (CaCC) are widely distributed in epithelial tissues, we tested adding the adenylyl cyclase agonist Fsk combined with the phosphodiesterase inhibitor IBMX to elevate cytoplasmic cAMP to stimulate CFTR and of adding UTP to elevate cytoplasmic Ca^{2+} to stimulate a CaCC, respectively (Fig. 3, *B–G*). Apparently distinct Cl^- conductances were activated since the purinergic agonist (UTP) and the β-adrenergic agonists (cAMP/IBMX) evoked distinctly opposite effects on the transepithelial potential in the presence of bilateral 150 mM NaCl Ringer. That is, UTP, the purinergic agonist, hyperpolarized, while cAMP/IBMX, the adrenergic agonist, depolarized the transepithelial potential by ~2.3 and 1.0 mV, respectively ($n = 5$; Fig. 3).

Constitutive and activated HCO_3^- secretion. In bilateral 25 mM $NaHCO_3^-$/125 mM NaGlu Ringer solution with luminal amiloride, the cAMP-mediated agonists Fsk/IBMX stimulated HCO_3^- secretion as shown by a significant hyperpolarization of V_t from a constitutive -2.3 ± 0.3 mV to a stimulated -5.5 ± 0.6 mV ($P < 0.01$, $n = 8$) along with a corresponding significant increase in I_{sc}^{eq} from a constitutive 16.8 ± 3.7 μA/cm² to stimulated 45.7 ± 8.7 μA/cm² ($P < 0.001$, $n = 8$), which was markedly inhibited by the CFTR inhibitor GlyH-101 when applied to the lumen; that is, V_t decreased from -5.5 ± 0.6 to -2.0 ± 0.5 mV ($P < 0.001$; $n = 8$) and I_{sc}^{eq} decreased significantly from 45.7 ± 8.7 to 13.2 ± 4.7 μA/cm² ($P < 0.002$, $n = 8$; Fig. 4, *A–D*).

Moreover, under the same conditions, the Ca^{2+}-mediated P2Y2 purinergic agonist UTP hyperpolarized V_t from a constitutive -2.0 ± 0.4 mV to a stimulated -6.0 ± 0.3 mV ($P <$

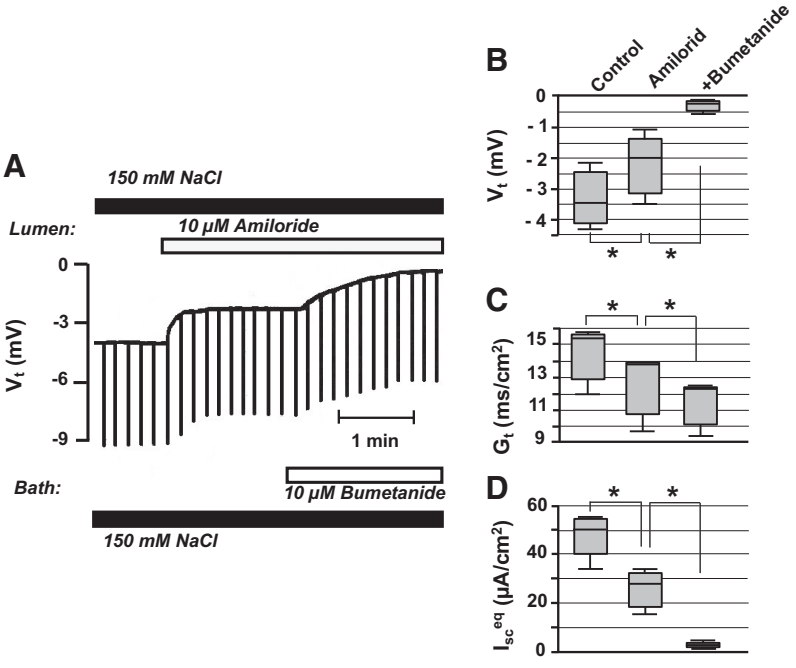

Fig. 2. Effect of inhibiting of absorption and secretion in human native small airway epithelia in bilateral 150 mM NaCl. *A*: representative trace of effects of luminal addition of amiloride (10 μM). Transepithelial potential (V_t) depolarized, transepithelial conductance (G_t), and equivalent short circuit current (I_{sc}^{eq}) decreased significantly. Subsequent addition of bumetanide (10 μM) to the serosal bath significantly decreased G_t and almost abolished V_t and I_{sc}^{eq}. *B–D*: box plots of data showing the effects of amiloride and bumetanide on V_t (*B*), G_t (*C*), and I_{sc}^{eq} (*D*). *$P <$ 0.05; $n = 3$. Plot parameters as in Fig. 1.

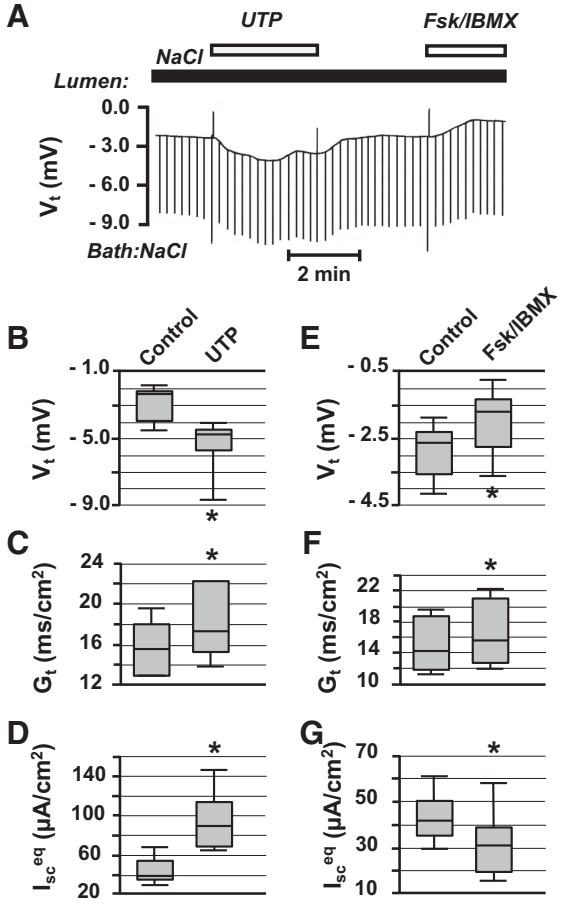

Fig. 3. Effects of agonists on transepithelial ion transport in human native small airway. *A*: a representative trace of transepithelial potential (V_t) showing effects of Ca^{2+}-mediated agonist UTP, cAMP-mediated agonists forskolin (Fsk)/3-isobutyl-1-methylxanthine (IBMX), and inhibitor GlyH-101 in bilateral 150 mM NaCl Ringer solutions on the small airway. The purinergic agonist UTP significantly hyperpolarized V_t. *B–D*: on the other hand, cAMP agonist Fsk/IBMX significantly depolarized V_t. *E–G*: box plots of data showing the effects of Ca^{2+}-mediated purinergic agonist UTP and cAMP-mediated agonists Fsk/IBMX in bilateral 150 mM NaCl on V_t (*B* and *E*), transepithelial conductance (G_t; *C* and *F*), and equivalent short circuit current (I_{sc}^{eq}; *D* and *G*). Purinergic (UTP) and β-adrenergic (cAMP) agonists evoke distinctly opposite effects on V_t and I_{sc}^{eq} ($n = 5$). Plot parameters as in Fig. 1. *$P < 0.05$; $n = 8$.

0.001, $n = 6$) accompanied by an increase in I_{sc}^{eq} from a constitutive 11.0 ± 2.3 μA/cm^2 to a stimulated 40.0 ± 4.8 μA/cm^2 ($P < 0.002$, $n = 6$). Both spontaneous and stimulated HCO_3^- secretion were almost completely inhibited by the CaCC inhibitor NFA when applied to the lumen, which decreased the constitutive V_t to -0.5 ± 0.3 mV ($P < 0.0001$; $n = 6$) and I_{sc}^{eq} to 3.2 ± 2.3 μA/cm^2 ($P < 0.0002$, $n = 6$; Fig. 5).

Additive effects of cAMP and Ca^{2+}-mediated HCO_3^- secretion. In the presence of luminal amiloride, the addition of luminal Fsk/IBMX to activate CFTR hyperpolarized V_t from -2.4 ± 1.0 to -5.6 ± 1.0 mV ($P < 0.01$, $n = 3$) and increased I_{sc}^{eq} from 15.4 ± 7.4 to 42.7 ± 15.1 μA/cm^2 ($P < 0.05$, $n = 3$). Subsequent luminal addition of UTP to activate a CaCC significantly hyperpolarized V_t even further to -7.9 ± 1.0 mV ($P < 0.02$, $n = 3$) and increased I_{sc}^{eq} to 60.5 ± 15.7 μA/cm^2 ($P < 0.01$, $n = 3$; Fig. 6).

DISCUSSION

Despite an abundant literature on ventilation dynamics and respiratory function, our understanding of the fluid and electrolyte transport mechanisms that determine the volume and composition of ASL in human small airways is limited. Since normal airway hygiene and pulmonary health depend crucially on ASL volume and HCO_3^- (40, 48), we sought to determine whether separate populations of cells coexist in human native small airway epithelia that would support simultaneous secretion and absorption of the ASL. In addition, we sought to determine whether the small airway epithelium also secretes bicarbonate physiologically.

Spontaneous transepithelial properties of electrolyte transport. To facilitate measurements of V_t, G_t, and I_{sc}^{eq} of small airways on the order of 2 mm ϕ, we modified a microperfusion device (12) to be able to use a glass capillary as the mucosal compartment of a miniature Ussing chamber (50) with which we measured the transepithelial electrolyte transport properties of very small, friable specimens of dissected native small airways of humans. In bilateral NaCl-Ringer solutions, the unstimulated small airway epithelia exhibited a small, lumen-negative electrical potential of about -3.3 ± 0.4 mV ($n = 21$), consistent with spontaneous values reported for small airways elsewhere (1, 4, 8, 50, 58, 60). This voltage derived from a mean active electrolyte transport equivalent current of 55.2 ± 11.4 μA/cm^2 ($n = 21$) through a mean conductance of 16.5 ± 2.2 mS/cm^2 ($n = 21$) (Table 1; Figs. 1–3). It seems unlikely that the small V_t is the result of tissue trauma, leaks, or edge damage, since the bi-ionic Cl^-:gluconate diffusion potential hyperpolarized V_t more than eightfold (Table 1; Fig. 1), and further, we discarded tissues that did not spontaneously exhibit a V_t of at least -20 mV lumen negative in the presence of a 150 mM Cl^- transepithelial gradient. In comparison, the electrophysiological parameters for human airways are similar to those reported for pig airways (Table 1) and are consistent with previous findings that the epithelium is anion selective (4, 50, 58). As a caveat, given the delicate nature of the tissue, the parameters measured may underestimate the in vivo activity of undisturbed, native tissue. Nonetheless, it seems reasonable that the observed responses to agonists and inhibitors accurately reflect the underlying physiological properties and behavior of the tissue.

Concurrent absorption and secretion. Amiloride blocks epithelial sodium channel-dependent Na^+ absorption (7). Herein, luminal amiloride significantly reduced G_t, depolarized V_t, and decreased I_{sc}^{eq}. Subsequently, adding the Na^+-K^+-$2Cl^-$ cotransporter inhibitor bumetanide to the bath almost abolished the spontaneous V_t and I_{sc}^{eq} (Fig. 2), indicating constitutive and concurrent Na^+ absorption and Cl^- secretion activities as seen in porcine airways (50). Moreover, since CFTR and CaCC channels are anion channels that support epithelial fluid transport, we selectively tested for cAMP-mediated stimulation of CFTR with Fsk/IBMX and for Ca^{2+}-mediated stimulation of CaCC with UTP as agonists. In human, as in porcine small airways, activating anion/Cl^- conductance with a β-adrenergic (cAMP) or a purinergic agonists (UTP) evoked distinctly opposite effects on the V_t in bilateral isotonic NaCl solutions (Fig. 3).

Recently, Benedetto et al. (5) reported that the tissue specific knockout of the TMEM16A gene in mouse intestine and

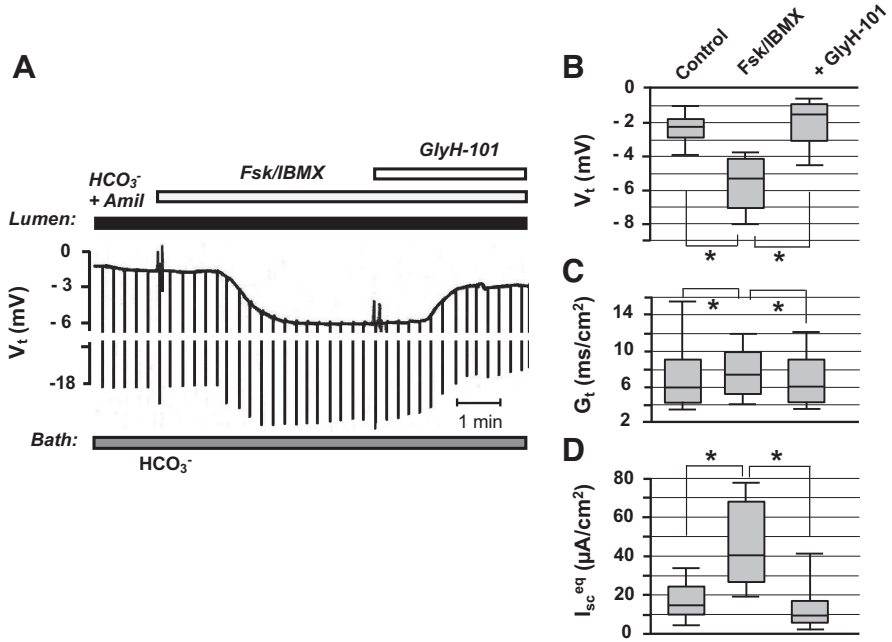

Fig. 4. Effect of cAMP agonist forskolin (Fsk)/3-isobutyl-1-methylxanthine (IBMX) and inhibitor GlyH-101 on HCO_3^- transport. *A*: representative trace of electrogenic properties HCO_3^- transport in human native small airways in bilateral 25 mM HCO_3^- Ringer solution (Cl^- free + luminal amiloride). Luminal addition of Fsk/IBMX significantly hyperpolarized transepithelial potential (V_t) and stimulated equivalent short circuit current (I_{sc}^{eq}). Luminal addition of CFTR inhibitor GlyH-101 blocked the cAMP response. *B–D*: box plots showing median, upper, and lower quartiles and whiskers showing upper and lower extreme data points of the effects of Fsk/IBMX, GlyH-101 on V_t (*B*), transepithelial conductance, G_t (*C*), and I_{sc}^{eq} (*D*). *$P < 0.05$; $n = 8$. Plot parameters as in Fig. 1.

airways not only eliminates CaCC but also abolished CFTR-mediated Cl^- secretion. They also reported that the complete TMEM16A knockout tissue did not cause an overt phenotype (5). Generally, mouse airways lacking TMEM16A did not show any mucus accumulation, which may support the concept that airway Na^+ absorption is physiologically more relevant than Cl^- secretion in mouse airways. Furthermore, Reddy and Quinton (41) did not see any evidence of CaCC in the sweat duct. However, the secretory coil has both CFTR and CaCC in β-adrenergic-sensitive cells. They further found that complete removal of Ca^{2+} had little effect on cAMP activation of CFTR in β-adrenergic-sensitive cells of secretory coil or on the ATP/cAMP activation of CFTR in permeablized duct cells (43). Therefore, the observations of Benedetto et al. (5) may be tissue specific. Furthermore, there is some controversy regarding the specific effect of the CaCC inhibitor NFA. Scott-Ward et al. (47) report that CFTR Cl^- channel is blocked by NFA but at a high concentration (>200 μM). Herein, we used half that concentration to block CaCC-dependent HCO_3^- secretion, which may have also blocked CFTR-dependent HCO_3^- secretion, since NFA alone blocked almost all I_{sc}^{eq} (Fig. 5); however, since the effects of UTP and IBMX were distinct and additive, we surmise that both CFTR and CaCC contribute to the HCO_3^--dependent I_{sc}^{eq}.

Depolarization of the transepithelial potential is consistent with increased Cl^- influx across the apical membrane from the lumen in the absorptive direction down a Cl^- electrochemical

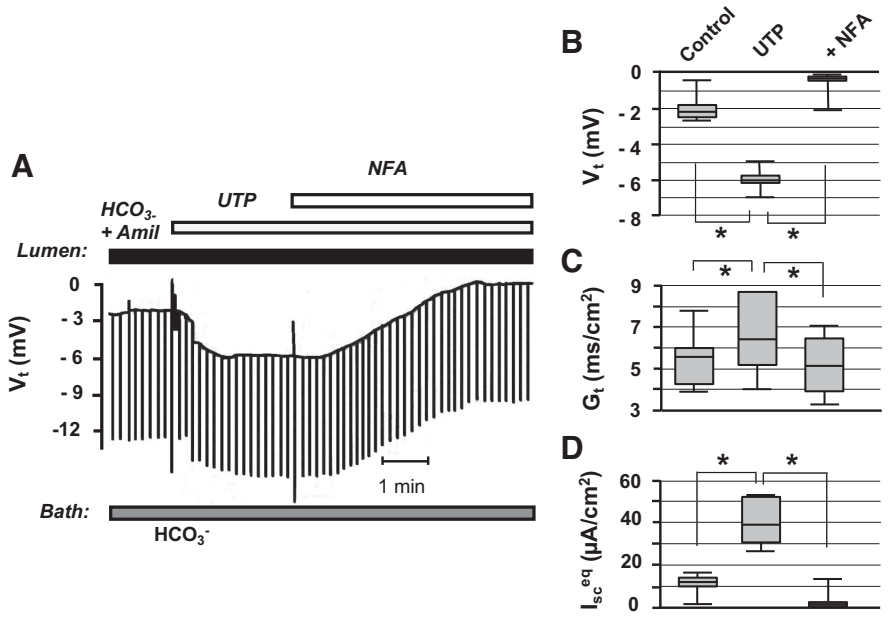

Fig. 5. Effect of Ca^{2+}-mediated agonist UTP and inhibitor niflumic acid (NFA) on HCO_3^- transport. *A*: representative trace of electrogenic properties of HCO_3^- transport in human native small airways in bilateral 25 mM HCO_3^- (Cl^- free + luminal amiloride). In the presence of luminal amiloride, luminal addition of UTP hyperpolarized transepithelial potential (V_t) and stimulated equivalent short circuit current (I_{sc}^{eq}). Luminal addition of Ca^{2+}-mediated inhibitor NFA blocked the I_{sc}^{eq} response. *B–D*: box plots of data showing the effects of UTP and NFA on V_t (*B*), transepithelial conductance (G_t; *C*), and I_{sc}^{eq} (*D*). *$P < 0.05$; $n = 6$. Plot parameters as in Fig. 1.

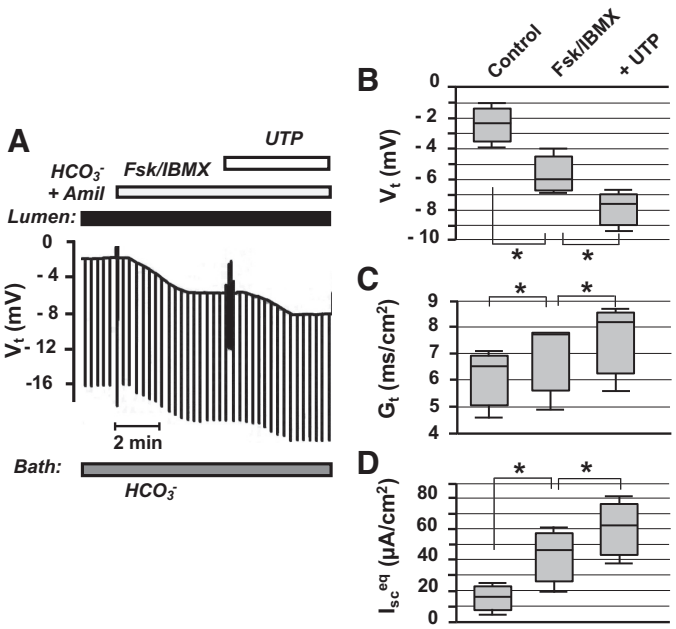

Fig. 6. Additive effects of cAMP and Ca^{2+}-mediated agonists on HCO_3^- transport. *A*: representative trace of electrogenic properties HCO_3^- transport in human native small airways with 25 mM HCO_3^- in both bath and lumen (Cl^- free + luminal amiloride). In the presence of luminal amiloride, luminal addition of cAMP-mediated agonists forskolin (Fsk)/3-isobutyl-1-methylxanthine (IBMX) hyperpolarized transepithelial potential (V_t) and stimulated equivalent short circuit current (I_{sc}^{eq}) Subsequent luminal addition of Ca^{2+}-mediated agonist UTP hyperpolarized V_t further, and increased I_{sc}^{eq}. *B–D*: box plots of data showing the additive effects of Fsk/IBMX and UTP on V_t (*B*), G_t (*C*), and I_{sc}^{eq} (*D*). $^*P < 0.05$; $n = 3$. Plot parameters as in Fig. 1.

gradient into cells that absorb Cl^-. In contrast, hyperpolarization of transepithelial potential is consistent with an increased efflux of Cl^- across the apical membrane into the lumen down an electrochemical gradient from cells that secrete Cl^-. Thus cAMP appears to predominantly stimulate cells with Cl^- channels (CFTR) that absorb anions from the lumen to cell, while UTP appears to predominantly stimulate cells with purinergic receptors that CaCC and secrete anions from the cell to lumen. Furthermore, it is important to note that transepithelial potential changes could also reflect changes in other ion conductance that are integral to overall transepithelial ion transport. For example, an increase basolateral K^+ conductance during transepithelial Cl^- secretion (42) with purinergic stimulation likely contributes to or supports the hyperpolarizarion of V_t during purinergic stimulation. However, the lack of effect of either cAMP or UTP on V_t in the presence of CFTR or CaCC inhibitors (Figs. 4 and 5) indicates that neither of these agonists directly affects basolateral K^+ conductance and therefore would not be expected to influence the observed changes in V_t. These results are consistent with a model in which small airways are composed of morphologically separated secretory and absorptive cells that function independently, concurrently, and continuously to maintain an appropriate ASL volume and airway hygiene (15, 50).

HCO_3^- secretion. Although airways have not been considered to be a HCO_3^--secreting tissue, indirect evidence that the human airways secrete HCO_3^- derives from the fact that pathologically affected organs in cystic fibrosis exhibit severely depressed or absent HCO_3^- secretion (3, 19, 21, 37, 38, 51). The CFTR protein in CF is localized along the apical border of ciliated airway epithelial cells (26, 58) and is best known for Cl^- conductance, but it also conducts HCO_3^- (28, 34, 45) so that the morbidity and mortality from respiratory pathology in CF are consistent with a failure of HCO_3^- secretion in small airways (55, 61). The data (Table 1) herein provides strong evidence that, like porcine (49), human native small airways physiologically secrete HCO_3^- constitutively and are under regulatory control as well.

cAMP and Ca^{2+}-mediated HCO_3^- secretion. Since CFTR is activated via increases in intracellular cAMP concentrations and mutations in CFTR cause cystic fibrosis (17, 20, 39, 44), we first tested the effects of directly elevating intracellular cAMP with Fsk/IBMX, which more than doubled the constitutive HCO_3^- I_{sc}^{eq}, and was effectively inhibited by the CFTR-selective inhibitor, GlyH-101 (30) (Fig. 4). These responses indicate a CFTR-dependent cAMP-mediated HCO_3^- secretion in the human small airways.

Cell culture studies suggest that the Cl^- conductance properties of CaCC are augmented in response to mutations in CFTR, which may imply shared roles for CFTR and CaCC channels in vivo (54). Furthermore, stimulation of CaCC is reported to increase mucus secretion, ciliary beat frequency, and inhaled particulate clearance (6, 25, 46). A Cl^- conductance independent of CFTR appears to be augmented in the tracheal epithelial cells cultured from CF mice (54), suggesting that CaCC may mitigate the CFTR defect. Studies in cultures of tracheal epithelial cells suggest significant roles for CaCC in regulating ASL volume, modulating mucus secretion (22), and responding to stressors such as cigarette smoke (23), lipopolysaccharide exposure, and inflammatory cytokines (32). We therefore tested the effect of adding the Ca^{2+}-mediated purinergic P2Y2 agonist UTP to the luminal bath, which significantly stimulated HCO_3^- I_{sc}^{eq} (Fig. 5). Applying luminal NFA to inhibit CaCC, not only blocked the stimulated current, but also decreased the total constitutive I_{sc}^{eq} indicating that basal, constitutive HCO_3^- secretion is not through CFTR but depends on another channel and implies that CFTR may be crucial for stimulated responses to stress as likely occurs in response to airway debris, infection, and/or inflammation.

Concurrently stimulating with both cAMP- and Ca^{2+}-mediated agonists together additively increased HCO_3^- I_{sc}^{eq} more than either agonist alone, further indicating that HCO_3^- can be secreted by separate components independently. Thus managing these separate responses therapeutically may enhance normal mucus formation and airway clearance as well as help correct underlying defects due to abnormally low pH in CF (9, 10) (Fig. 6).

In summary, human native small airways appear to consist of separate groups of secretory and absorptive cells that locally control luminal surface fluid to maintain appropriate airway hydration. Human small airways constitutively secrete HCO_3^- and are capable of significantly increasing HCO_3^- secretion via two apparently separate pathways, one dependent on CFTR, and another dependent on a CaCC. Physiological regulation of HCO_3^- secretion is critical for discharge of mucin as well as for innate defenses of airways including bactericidal activity of antimicrobial peptides (37, 53). Since failure to secrete HCO_3^- results in poor mucociliary and pathogen clearance, stimulating

HCO_3^- secretion therapeutically may be a strategic target for cystic fibrosis and possibly other airway diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.K.M.S. and P.M.Q. conceived and designed research; A.K.M.S. performed experiments; A.K.M.S. analyzed data; A.K.M.S. and P.M.Q. interpreted results of experiments; A.K.M.S. prepared figures; A.K.M.S. drafted manuscript; A.K.M.S. and P.M.Q. edited and revised manuscript; A.K.M.S. and P.M.Q. approved final version of manuscript.

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