Acid secretion and proton conductance in human airway epithelium

HORST FISCHER, JONATHAN H. WIDDICOMBE, AND BEATE ILLEK
Children’s Hospital Oakland Research Institute, Oakland, California 94609

Received 2 August 2001; accepted in final form 8 November 2001

Acid secretion and proton conductance in human airway epithelium. Am J Physiol Cell Physiol 282: C736–C743, 2002.—Acid secretion and proton conductive pathways across primary human airway surface epithelial cultures were investigated with the pH stat method in Ussing chambers and by single cell patch clamping. Cultures showed a basal proton secretion of 0.17 ± 0.04 μmol·h⁻¹·cm⁻², and mucosal pH equilibrated at 6.85 ± 0.26. Addition of histamine or ATP to the mucosal medium increased proton secretion by 0.27 ± 0.09 and 0.24 ± 0.09 μmol·h⁻¹·cm⁻², respectively. Addition of mast cells to the mucosal medium of airway cultures similarly activated proton secretion. Stimulated proton secretion was similar in cultures bathed mucosally with either NaCl Ringer or ion-free mannitol solutions. Proton secretion was potently blocked by mucosal ZnCl₂ and was unaffected by mucosal bafilomycin A₁, Sch-28080, or ouabain. Mucosal amiloride blocked proton secretion in tissues that showed large amiloride-sensitive potentials. Proton secretion was sensitive to the application of transepithelial current and showed outward rectification. In whole cell patch-clamp recordings a strongly outward-rectifying, zinc-sensitive, depolarization-activated proton conductance was identified with an average chord conductance of 9.2 mS/pF (at 0 mV and a pH 5.3-to-pH 7.3 gradient). We suggest that inflammatory processes activate proton secretion by the airway epithelium and acidify the airway surface liquid.

THE MUCOSA OF THE AIRWAY SURFACE epithelium is lined with a thin layer of fluid called the airway surface liquid (ASL). The composition of the ASL affects its physiological functions, the most important of which are removal of inhaled particles and antimicrobial activity (36). Active transport of Na⁺ and Cl⁻ by the airway epithelium have been well characterized and recognized as critical determinants of ASL composition and depth. In contrast, the regulation of the H⁺ concentration in the ASL has received little attention. Acidic luminal pH has been shown to inhibit ciliary beating (4) and to cause bronchoconstriction (1), cough (39), loosening of the epithelial cells from one another (13), and detachment from the basement membrane (17). Acidic ASL has been implicated in airway diseases such as asthma (19, 29) and cystic fibrosis (CF) (33). Although the airway epithelium has been shown to secrete HCO₃⁻, normal ASL in ferret trachea was previously reported as slightly acidic (pH 6.85) compared with plasma (26). In human primary airway cultures a pH of 6.9 was recently found with the use of either pH-selective microelectrodes (5) or a pH-sensitive fluorophore (22).

The concentration of HCO₃⁻ is one determinant of the pH of ASL, and active secretion of HCO₃⁻ across primary airway cultures or cell lines has been demonstrated (28, 33), presumably reflecting the HCO₃⁻ permeability of the apical CF transmembrane conductance regulator (CFTR) Cl⁻ channel (31). Jayaraman et al. (22) estimated a HCO₃⁻ concentration of 8 mM in the ASL from their measurements of pH and a partial pressure of CO₂ of 3 kPa. With that HCO₃⁻ concentration and with an average CO₂ level in the airways in vivo of 2.2 kPa, the ASL is predicted to be slightly alkaline (pH ~7.3), which is in contrast to the reported relative acidity of ASL. Thus we hypothesized that the airway epithelium expresses a regulated mechanism to secrete H⁺ into the ASL. Because the volume of the ASL is small (~1 μl/cm²) it can be expected to be rapidly acidified by cellular H⁺ transport. Recently, Hunt et al. (19) found that the exhaled breath of asthmatic patients during an attack is markedly acidic (pH 5.2). We hypothesized that during an inflammatory challenge the airway epithelium secretes increased amounts of acid into the ASL. In this report we measured acid secretion by human airway epithelial cultures in vitro and investigated its mechanism and regulation. We conclude that mucosal histamine, ATP, or mast cells activate epithelial acid secretion that is mediated mainly by an electrogenic apical proton conductance.

METHODS

Cell cultures. Human tracheal primary cultures were isolated and cultured as previously described (40). In brief, strips of epithelium were removed from the underlying tissues and treated with protease overnight. The resulting isolated, dispersed cells were plated on permeable filter sup-

Address for reprint requests and other correspondence: H. Fischer, Children’s Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609-1673 (E-mail: hfischer@chori.org; http://www.chori.org/scientists/fischer.html).

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ports (Snapwell, 0.4-μm pore size, 1-cm² area; Corning Costar, Cambridge, MA) precoated with human placental collagen (15 μg/cm²) at a density of ~10⁶ cells/cm². Cells were grown in DMEM-F12 culture medium supplemented with 2% Ultros G (Biotechnics, Paris, France) and antibiotics. Transwell sheets were grown to confluence in an air-liquid interface in a tissue culture incubator gassed with 5% CO₂ and air. The cystic fibrosis JME/CF15 airway cell line (23) was cultured in a DMEM-F-12 mixture supplemented with 10% fetal bovine serum and (per ml) 5 μg of insulin, 0.5 μg of hydrocortisone, 10 ng of epidermal growth factor, 5 ng of transferrin, 1.3 ng of triiodothyronine, 43 ng of adenine, and 1 μg of epinephrine. For patch clamping, cells were seeded at low density on cover glasses. The human mast cell line HMC-1 was kindly provided by Dr. J. H. Butterfield and was cultured as described previously (2).

Quantification of proton secretion. Proton secretion was measured with the pH stat titration method in an Ussing chamber (14). Cultures (1-cm² exposed area) were mounted in an Ussing chamber (Physiologic Instruments, San Diego, CA) and, unless otherwise described, bathed serosally with HEPES-buffered solution and mucosally with buffer-free solution (3 ml each). Solutions were constantly gassed with oxygen and were nominally free of HCO₃⁻. The pH of the mucosal-methyl-D-glucamine (NMDG). In some experiments the solution (3 ml each). Solutions were constantly gassed with oxygen and were nominally free of HCO₃⁻. The pH of the mucosal side was set to 7.3 to prevent air CO₂ from entering the solutions. Standard NaCl Ringer solutions contained (in mM) 140 NaCl, 2 KCl, 15 glucose, 2 CaCl₂, and 1 MgCl₂ (mucosal) or 140 NaCl, 2 KCl, 5 glucose, 10 HEPES, 2 CaCl₂, and 1 MgCl₂ (serosal), adjusted to pH 7.3 with N-methyl-D-glucamine (NMDG). In some experiments the serosal solution was HCO₃⁻/CO₂, buffered containing (in mM) 120 NaCl, 25 NaHCO₃, 2 KCl, 5 glucose, 2 CaCl₂, and 1 MgCl₂, gassed with 5% CO₂-95% O₂. The pH of the mucosal solution was continuously measured, amplified, and recorded on a computer through a 12-bit analog-to-digital converter. Mucosal pH was manually titrated to a target value of pH 7.3 with 10 mM NaOH made from certified standards. Volumes on the order of 0.5–2.0 μl were added with a precision pipette (Eppendorf Ultra Micro 2.5; Brinkmann Instruments). From the added volume and the time between identical pH readings in the recording the rate of H⁺ secretion (Jₜₚ) was determined. Rates are expressed in micromoles per hour per square centimeter, and positive rates refer to acidification of the mucosal medium. To test the quality of our measurements and to identify the cultures as the source of the measured mucosal acidity we did control experiments without cells for all conditions. No component of our recording system released significant acid equivalents, and pH was stable over the course of several hours. Tissues were investigated under current-clamped conditions. Unless otherwise described, current was clamped to zero. Transepithelial potential (Vₑ, referenced to the serosal side) was continuously recorded, and transepithelial resistance (Rₑ) was determined from 20-μA pulses. Positive transepithelial currents refer to cation secretion into the mucosal bath. Measurements were done at 35–37°C.

Whole cell patch-clamp recordings. Cells were whole cell patch-clamped after 2 days in culture as previously described (20) on a temperature-controlled stage (35–37°C) of an inverted microscope. Cells were bathed in (in mM) 100 HEPES, 85 gluconic acid, 100 NMDG, 2 Ca gluconate, 1 Mg gluconate, and 10 glucose, pH 7.3. The pipette was filled with (in mM) 2-(N-morpholino)ethanesulfonic acid (MES), 81.6 gluconic acid, 70 NMDG, 10 NMDG-EGTA, 1 glucose, 1 MgCl₂, 3.3 Mg-ATP, and 0.07 Li-GTP, pH 5.3. The bath electrode was made with the pipette-filling solution (but ATP/GTP free) and a 3% agar bridge. Junction potentials were measured, zeroed, and carefully observed for stability. With these solutions, pipette resistance was ~5 MΩ. Only seals >50 GΩ were used for recordings. The access resistance (Rₐ) and the cell membrane capacitance (Cₘ) were determined by fitting the current transients caused by a 10-mV voltage pulse with a single exponential. Measured Rₐ was 15.4 ± 1.8 MΩ (n = 13), and Cₘ was 29.1 ± 4.3 pF. Current-voltage step protocols from ~80 mV to +60 mV were applied, and resulting step currents were recorded. Whole cell conductance was calculated as the chord conductance at 0 mV. When the specific membrane conductance (Gₘ in pS/pF) was calculated, whole cell conductance was corrected for Rₑ and normalized to Cₘ.

Drugs. Stock solutions of histamine (free base, 10 mM), ATP (Na salt, 50 mM), and ZnCl₂ (1 mM) were prepared in NaCl Ringer solution, and pH was adjusted to 7.3. Amiloride stock was prepared at 10 mM in water, and pH was adjusted to 7.3. 3-(Cyanomethyl)-2-methyl-8-(phenylimethoxy)-imidazo-[1,2a]-pyridine (Sch-28080) was kindly provided by Dr. T. E. Machen (Univ. of California, Berkeley) and prepared as a 5 mM stock in ethanol. Bafilomycin A₁ (500 μM) and ouabain (100 mM) were prepared as stocks in dimethyl sulfoxide.

Statistics. Data are given as original values or as means ± SE; n is the number of cultures tested. Unpaired t-tests were used to test for statistical difference (P < 0.05) between means, and one-sample t-tests were used to test whether responses were significantly different from zero.

RESULTS

Proton secretion across human airway cultures was investigated in HCO₃⁻/CO₂-free solutions in Ussing chambers. At a mucosal pH of 7.3 all tested cultures acidified the mucosal medium. In NaCl solutions the basal rate of acidification was Jₜₚ = 0.17 ± 0.04 μmol·h⁻¹·cm⁻² (n = 26). Basal Vₑ was −15.6 ± 4.1 mV, and Rₑ was 1,016 ± 137 Ω·cm². Figure 1 shows an example of a measurement of mucosal pH and determination of the basal rate of acidification. Initially, the pH was titrated two times with NaOH to ~7.3 to calculate the rate of acidification. When no NaOH was added, the pH reached an equilibrium at 6.85 ± 0.26 (n = 5) after 41 ± 9 min.

Addition of histamine or ATP (100 μM each) to the mucosal bath potently stimulated H⁺ secretion (Fig. 2, A and B). Both agents typically caused a fast initial increase in pH.
rise followed by a slow decay of $J_H$. Figure 2C shows the average peak effects of mucosal addition of histamine and ATP. In contrast, when histamine or ATP was added to the serosal bath, $J_H$ was unchanged (not shown). We also tested 50 μM serotonin and 20 μM forskolin, which showed no significant effects on $J_H$ [serotonin, $+0.04 \pm 0.05$ μmol·h$^{-1}$·cm$^{-2}$ (n = 3); forskolin, $+0.01 \pm 0.10$ μmol·h$^{-1}$·cm$^{-2}$ (n = 3)].

To identify the mechanism(s) in the apical membrane responsible for the $H^+$ secretion we tested known blockers of $H^+$ transporters. Cultures were stimulated with a combination of histamine and ATP, which we found to result in more sustained and stable responses compared with application of the single agonists. Combined treatment resulted in peak responses of $0.59 \pm 0.06$ μmol·h$^{-1}$·cm$^{-2}$ (n = 13). We tested the effects of the following blockers: 5 μM Sch-28080 to probe for the gastric-type $K^+$-$H^+$-ATPase, 100 μM ouabain to probe for the non-gastric-type $K^+$-$H^+$-ATPase, 100 nM bafilomycin A$_1$ to probe for the V-type H$_2$-ATPase, 400 μM amiloride to probe for the Na$^+$-$H^+$ exchanger, and 200 μM ZnCl$_2$ to block H$^+$ channels. All blockers were added to the mucosal side. Figure 2, D, E, and G, shows typical blocker experiments, and Fig. 2F summarizes the average effects of blockers. Both bafilomycin A$_1$ and Sch-28080 are highly specific and selective blockers. Neither showed significant effects on $J_H$ (effects not different from zero, 1-sample t-tests), indicating that the V-type H$_2$-ATPase and the gastric-type K$^+$-H$^+$-ATPase do not significantly contribute to $H^+$ secretion across airways. Similarly, mucosal ouabain (Fig. 2, G and F) showed very small effects that were not
Addition of amiloride to the mucosal bath had variable effects on $H^+$ secretion. In 4 of 11 cultures tested $J_{H}$ was transiently inhibited by $-0.17 \pm 0.07 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$ (peak inhibition), corresponding to an inhibition of 34% of total $H^+$ secretion. Figure 2D shows an example. In the other seven cultures tested amiloride had no significant effect ($0.02 \pm 0.03 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$). In Fig. 2F the total average effect of amiloride is given. Interestingly, the cultures that showed an amiloride-sensitive $J_{H}$ also expressed a larger amiloride-sensitive $V_{i}$. In these cultures amiloride blocked 76% ± 19% of $V_{i}$, whereas in the cultures that did not show an amiloride-sensitive $J_{H}$, $V_{i}$ was blocked by only 17% ± 8%. This suggested that the effects of amiloride on $J_{H}$ were likely not mediated by an apical Na$^+$/H$^+$ exchanger. However, the data are consistent with the notion that the activity of apical Na$^+$ channels affected $J_{H}$ (see discussion).

$ZnCl_2$ (200 $\mu$M) added to the mucosal bath caused sustained block of $J_{H}$ (Fig. 2E and G). $ZnCl_2$ was the only blocker that consistently blocked a large fraction of $J_{H}$. In nine of nine cultures tested, 200 $\mu$M $ZnCl_2$ blocked on average 70% ± 8.3% of $J_{H}$. These data suggest that a zinc-sensitive $H^+$ conductance is the major mechanism for the transepithelial $J_{H}$ in airway cultures.

In addition, we measured $H^+$ secretion in cultures that were incubated on the mucosal side with nominally ion-free solution (300 mM mannitol) to determine the ion dependence of the apical mechanism. Serosal solution was standard NaCl-HEPES Ringer solution. Under these conditions the basal rate of acidification was $0.07 \pm 0.03 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$ ($n = 3$). Addition of ATP to the mucosal bath resulted in an increase of $J_{H}$ of $0.28 \pm 0.02 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$. An example is shown in Fig. 2H. Addition of histamine resulted in peak increases of $J_{H}$ of $0.18 \pm 0.03 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$ ($n = 2$), and histamine plus ATP resulted in increases of $0.68 \pm 0.08 \, \mu$mol$\cdot$h$^{-1}\cdot$cm$^{-2}$ ($n = 2$). The rates measured in ion-free solutions were not different from the rates obtained in NaCl Ringer solutions.

The zinc sensitivity and the ion independence of stimulated $H^+$ secretion indicated an apical $H^+$ conductance. Therefore, $J_{H}$ should be dependent on the electrochemical driving force across the apical membrane. To test this hypothesis transepithelial currents were passed across the epithelium. Airway cultures were bathed in NaCl Ringer, and transepithelial current was clamped for 10 min sequentially to $-200, 0,$ or $+200 \, \mu$A/cm$^2$ before and after ATP stimulation. Figure 3 shows $J_{H}$ measured at different currents. It should be noted that $J_{H}$ was positive at all clamped currents. Positive currents (which depolarize the apical membrane) significantly increased $J_{H}$. The inhibition of $J_{H}$ by negative currents (which hyperpolarize the apical membrane) was less than the observed stimulation by positive currents. This relation suggested outward rectification of $H^+$ currents with respect to the apical membrane potential.

To measure $H^+$ currents directly, single JME airway cells were whole cell patch-clamped under conditions selective for $H^+$ currents (see methods). For the patch clamp experiments JME cells were used in preference to primary ciliated airway cells because of 1) the difficulty in obtaining high-resistance seals on ciliated cells and 2) the poor space clamping of the apical membrane of ciliated cells. $H^+$ currents were measured selectively under conditions adapted from the measurement of $H^+$ currents in alveolar cells (3), and cells were prestimulated with 100 $\mu$M ATP in the bath. $H^+$ currents were identified by 1) reversal potential, 2) voltage-dependent activation, and 3) sensitivity to zinc. Figure 4 shows typical whole cell recordings. In the presence of a pH 5.3-to-pH 7.3 proton gradient from cell to bath $H^+$ currents showed a negative reversal potential of $-72 \pm 6.1$ mV ($n = 13$). When membrane potential was stepped to depolarizing potentials, currents showed slow activation and strong outward rectification (Fig. 4A and B), which are typical characteristics of $H^+$ currents (3). On average, we found a steady-state $H^+$ conductance of $9.2 \pm 3.8 \, pS/pF$ ($n = 13$, chord conductance at 0 mV, with an average cell capacitance of 29 ± 4 pF). Figure 4C shows a whole cell patch-clamp experiment in which the effect of $ZnCl_2$ was tested. The membrane potential was clamped to $-80$ mV and pulsed to $+20$ mV every 10 s to continuously monitor the voltage activation of the $H^+$ current as an identif. Before addition of $ZnCl_2$, large currents activated during the depolarizing pulses. Addition of $ZnCl_2$ to the bath readily blocked the voltage-activated positive (outward) currents. Figure 4D shows details of the voltage-activated currents before and after addition of $ZnCl_2$. © American Physiological Society

Fig. 3. Proton secretion is affected by injected current. Constant currents were passed across the epithelium, and $H^+$ secretion into the mucosal compartment was measured. Data are from 3 tissues before (□) and after (●) ATP activation. For some points, SE bars are smaller than symbols.
In vivo the main source for mucosal histamine in the airways is probably mucosal mast cells. To test the direct effect of mast cells on H⁺ secretion by the airway epithelium we used the human mast cell line HMC-1 (2). HMC-1 cells constitutively degranulate and release histamine and other factors (2). HMC-1 cells were suspended in unbuffered NaCl Ringer, the pH was adjusted to 7.3, and a cell suspension containing 1.5 × 10⁶ HMC-1 cells was added to the mucosal compartment. Figure 5A shows a typical experiment. Addition of HMC-1 cells resulted in a large activation of H⁺ secretion. On average, J_H values of 0.87 ± 0.33 μmol·h⁻¹·cm⁻² (n = 3; peak value) were stimulated, which is a significantly larger response than treatment with histamine (0.27 ± 0.09 μmol·h⁻¹·cm⁻²; Fig. 2C). The increased acidification may have been caused by another factor released by the mast cells or by H⁺ release by the mast cells. This latter possibility was tested by adding HMC-1 cells to experiments without airway cultures (Fig. 5B). When added, 1.5 × 10⁶ HMC-1 cells showed a brief release of acid with a rate of 0.56 ± 0.028 μmol/h (n = 4 experimental runs). Within <10 min J_H from HMC-1 cells returned to zero, indicating that parts of the initial peak, but not the continuous acid secretion by airway epithelium, was caused by a brief acid release from HMC-1 cells.

We next performed measurements in the presence of 25 mM HCO₃⁻ and 5% CO₂ on the serosal side. Basal Vₜ was 22.7 ± 5.4 mV, and Rₜ was 730 ± 128 Ω·cm² (n = 7). The mucosal medium initially either acidified (5 of 7 cultures) or alkalinized (2 of 7 cultures). On average, basal J_H was 0.15 ± 0.28 μmol·h⁻¹·cm⁻² (not signif-
Acid secretion by human airways

We report here that human airway surface epithelium shows secretion of H⁺ into the airway lumen regulated by mucosal histamine or ATP. The effect of histamine suggests that the stimulation of H⁺ secretion in vivo involves mucosal mast cells, which we also show to elicit acid secretion. Thus from our data we propose that during airway inflammation, such as in asthma, in CF, or during allergic reactions, H⁺ secretion by the airways is activated and the ASL acidifies. In addition, ATP is released by cells under various conditions including airway epithelium during mechanical stress (18). Therefore, stimulants that are unrelated to the mast cell-mediated responses can also lead to acidification of the ASL.

Properties of channel-mediated H⁺ secretion in airways. We aimed to identify the type of H⁺ transporter that operates in the apical cell membrane of airways. We used drug sensitivity and voltage- and ion dependence to characterize the H⁺ transporter. Our data show that H⁺ secretion by airways occurred primarily via a zinc-sensitive apical membrane H⁺ conductance for the following reasons. 1) Under ion-free conditions or in the presence of ions rates of H⁺ secretion were similar. Therefore, the ion-dependent H⁺ transporters (i.e., the Na⁺/H⁺ exchanger and the K⁺/H⁺-ATPase) did not contribute significantly. 2) In transepithelial current-clamp experiments the application of positive (outward) current across the epithelium (which polarizes the cytoplasmic face of the apical membrane positively) was accompanied by an increase in H⁺ secretion. Current passed in the opposite direction reduced H⁺ currents, indicating that H⁺ secretion is electrogenic. 3) In whole cell recordings typical identifiers of H⁺ currents were found including strong outward rectification and activation by depolarization (3, 9, 25, 35). 4) ZnCl₂ effectively blocked H⁺ secretion in transepithelial recordings and in whole cell patch-clamp recordings. In transepithelial recordings Sch-28080, bafilomycin A₁, or ouabain had no significant effects on H⁺ secretion, indicating that the K⁺/H⁺-ATPase and the V-type H⁺-ATPase did not significantly contribute to transepithelial H⁺ secretion. Thus the results of the blocker experiments are consistent with the results obtained from the ion-free experiments. An apically localized H⁺ conductance in human airways was identified by its pharmacological and biophysical characteristics, and other transporters could be excluded by the observed ion independence of H⁺ secretion and by using blockers. Interestingly, in parallel experiments with bovine tracheal cultures we found a significant block of H⁺ secretion by bafilomycin A₁ (data not shown), indicating species-specific variations in H⁺ transporters in the airways.

Owing to the strong outward rectification of the H⁺ currents and the localization of the conductance to the apical membrane, this pathway is expected to support H⁺ fluxes in the secretory direction. This is consistent with our observation that only H⁺ secretion (and not H⁺ absorption) was measured in this report (see, for example, Fig. 3). Our data are consistent with the notion that an outward electrochemical H⁺ gradient exists across the apical membrane that drives H⁺ secretion across the airway epithelium. H⁺ conductances in other cell types have been shown to be extremely dependent on the membrane potential such that at negative potentials currents were very small but at depolarizing and positive potentials H⁺ currents activated. An additional critical feature is the activation of the H⁺ conductance by an inside-to-outside H⁺ gradient, as shown in detail for the H⁺ conductance in rat alveolar cells (8). In fact, in the absence of an H⁺ gradient the threshold voltage for activation was shown to be nonphysiologically high, current activation was slow, and the currents were very small (8). Thus, in cell types where H⁺ channels were found, their assumed function is the dissipation of high intracellular H⁺ concentrations during metabolic acidosis. For example, in phagocytic neutrophils the intracellular space near the membrane acidifies by release of H⁺ from NADPH during generation of superoxide anions by the membrane-bound NADPH oxidase, and H⁺ currents are driven by the metabolic acidosis at the membrane (15). Similarly, a H⁺ conductance in mast cells has been proposed to dissipate the stimulus-induced cytosolic acidification (25). However, in cultured human nasal airway cells cytosolic pH was reported to be 7.15 (30), which under physiological conditions would result in a H⁺ gradient from the ASL (pH = 6.9) into the cell. Because our data are consistent with an outward H⁺ gradient, we suggest that the intracellular pH near the apical membrane is markedly acidic in airway cells. This hypothesis relies on the following observations. Mucosal pH of airway cultures equilibrated at pH = 6.85 (Fig. 1). Furthermore, the apical membrane potential (Vₘ) in human airway cell cultures has been reported as −26 ± 1 mV (38) and −19 ± 2 mV (37). Assuming an average Vₘ of −22.5 mV, then at equilibrium the sum of the Nernst potential for H⁺ and
of the apical membrane is a significant determinant of $V_a$. Thus the measured inhibition of $H^+$ secretion by amiloride is well explained by an amiloride-induced hyperpolarization of $V_a$.

Role of acidic ASL in asthma. In asthma, the pH of condensed exhaled breath has been reported to be markedly acidic (19), a result that could reflect increased rates of acid secretion by the airway epithelium. Asthma is characterized by an increased number of luminal mast cells (12) that release their contents more readily than normal, and the levels of mast cell degranulation products in the ASL are correspondingly higher than normal (10). Thus we propose that increased levels of histamine (and possibly other mast cell products) in the lumen of the airways of asthmatic patients causes an acidification of the ASL. At the histamine-stimulated rates of $H^+$ secretion that we have found (0.41 μmol·h⁻¹·cm⁻²) and an estimated buffer capacity of the ASL of 40 mM/pH, the initial rate of acidification of the ASL can be calculated as 0.17 pH units/min. Acidic ASL then produces as yet undetermined functional changes in the airway epithelium that might initiate or exacerbate asthma attacks. Mast cells (1) secrete various factors in addition to histamine (11 and 2) that would stimulate epithelial $H^+$ channels and release $H^+$ (25). Therefore, we tested directly the effect of applying a suspension of the mast cell line HMC-1 to the mucosal surface of tracheal culture. The resulting $H^+$ secretion by the epithelium was significantly larger than that seen with histamine or ATP. However, mast cells alone showed a brief but significant release of acid (Fig. 5), suggesting that the continuously elevated $H^+$ secretion by the airway cultures was mediated by mast cell-derived factors.

Role of acid secretion in CF and bacterial colonization. Asthma and CF share some common symptoms, such as sinusitis and frequent respiratory infections. CF is caused by a mutation in the CFTR Cl⁻ channel. CFTR is also conductive for $HCO_3^-$ (31). In addition, Na⁺ absorption is increased in CF, resulting in a depolarization of $V_a$ (37). Both a reduced $HCO_3^-$ secretion and a depolarized $V_a$ (which would stimulate epithelial $H^+$ secretion) will decrease the pH of the ASL in CF. Preliminary measurements of the mucosal pH in noses of CF patients indeed showed more acidic values than normal (6.2 vs. 6.65; Ref. 7). Currently it is unclear whether the ASL acidity contributes to CF lung disease. However, it has been demonstrated that mucosal acidity affects the function of the epithelium. For example, prolonged treatment of airway epithelium with pH < 6.5 caused significant damage (13, 17). Compromised epithelial integrity, in turn, has been shown to cause increased bacterial binding (27). Thus it is possible that ASL acidification is a significant step during airway inflammation and infection in CF as well as asthma.

We thank Dr. Walter Finkbeiner (University of California, Davis) for providing the airway cultures, Dr. Terry Machen (University of California, Berkeley) for discussion, and Eric Wunderlich for the JME cell cultures.
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This work was supported by National Heart, Lung, and Blood Institute Grant 1-P50-HL-60288.

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