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Research Article

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Fluid and Electrolyte Transport by Cultured Human Airway Epithelia

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Abstract

An understanding of the fluid and electrolyte transport properties of any epithelium requires knowledge of the direction, rate, and regulation of fluid transport and the composition of the fluid. Although human airway epithelia likely play a key role in controlling the quantity and composition of the respiratory tract fluid, evidence for such a role is not available. To obtain such knowledge, we measured fluid and electrolyte transport by cultured human nasal epithelia. Under basal conditions we found that epithelia absorbed Na^+ and fluid; both processes were inhibited by addition of amiloride to the mucosal surface. These data suggest that active Na^+ absorption is responsible for fluid absorption. Interestingly, Na^+ absorption was not accompanied by the net absorption of Cl^- ; some other anion accompanied Na^+ . The combination of cAMP agonists and mucosal amiloride stimulated the secretion of NaCl -rich fluid. But surprisingly, the response to cAMP agonists in the absence of amiloride showed substantial intersubject variability: cAMP stimulated fluid secretion across some epithelia, for others, cAMP stimulated fluid absorption. The explanation for the differences in response is uncertain, but we speculate that the magnitude of apical membrane Na^+ conductance may modulate the direction of fluid transport in response to cAMP. We also found that airway epithelia secrete H^+ and absorb K^+ under basal conditions; both processes were inhibited by cAMP agonists. Because the H^+/K^+ -ATPase inhibitor, SCH 28080, inhibited K^+ absorption, an apical membrane H^+/K^+ -ATPase may be at least partly responsible for K^+ and H^+ transport. However, H^+/K^+ exchange could not entirely account for the luminal acidification. The finding that cAMP agonists inhibited luminal acidification may be explained by the recent finding that cAMP increases apical HCO_3^- conductance. These results provide new insights into how the intact airway epithelium may modify the composition of the respiratory tract fluid. (*J. Clin. Invest.* 1993. 91:1590–1597.) Key words: fluid transport • airway surface fluid • airway liquid • Na^+ absorption • Cl^- secretion • K^+ absorption • H^+ secretion • pH • anion gap • lactate

Introduction

Epithelia form a barrier that separates luminal from interstitial compartments. By secreting or absorbing solutes, they transport fluid across the epithelium and thereby modify the quan-

tity and composition of the fluid in the luminal compartment. In addition, epithelia respond to a variety of hormones, autoids, and neurotransmitters with a change in the rate of transepithelial fluid transport. Knowledge of the direction, rate, and regulation of fluid transport and the composition of the fluid on the luminal surface of an epithelium is central to understanding the function of that epithelium. Unfortunately, such knowledge is quite limited for airway epithelia. On the one hand, a number of studies have measured transepithelial electrolyte transport by the airway epithelia of several species (for review see reference 1). In addition, some of the individual ion transport processes have been studied in molecular detail. But on the other hand, our current knowledge of transepithelial fluid transport, its regulation, and its composition is clearly inadequate to understand the function of the airway epithelium.

The airway epithelium is covered by a blanket of mucus that traps inhaled particulate materials (2). Between this mucus blanket and the epithelium lies a thin, aqueous sublayer of fluid. From the epithelial surface, cilia project into the fluid so the tips of cilia make contact with the mucus blanket. The coordinate function of the mucus, fluid layer, and cilia is to propel mucus toward the oropharynx (mucociliary clearance) (3), thereby providing an important pulmonary defense mechanism. Electrolyte transport by airway epithelia is thought to be important for controlling the quantity and composition of the respiratory tract fluid. This notion is best supported by the observation that pulmonary disease occurs in patients with cystic fibrosis (CF),¹ a genetic disease characterized by defective Cl^- secretion and enhanced Na^+ absorption across airway epithelia (for review see reference 4).

The paucity of knowledge of the respiratory tract fluid from normal airway epithelium results in large part from its inaccessibility. Sputum has been collected and analyzed (5, 6); but sputum also contains mucus and may be modified by cellular debris, bacteria, and saliva, especially in subjects with increased respiratory secretions (7, 8). Airway surface fluid has been collected from dogs (9), although their use of the airway for evaporative cooling (panting) suggests the possibility that differences between canine and human airway surface fluid may exist. Sheep and human airway surface fluid have been collected in vivo and the electrolyte concentrations in the airway fluid measured (10, 11). However, this technique does not allow net ion transport rates and their regulation to be determined.

To better understand the physiology of the airway epithelium, we measured fluid and electrolyte transport across primary cultures of human nasal epithelia. We used cultured cells so that we could specifically study fluid transport by the surface epithelium and avoid contributions made by submucosal glands, mucus, and inflammatory cells. This approach also al-

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1. Abbreviations used in this paper: CF, cystic fibrosis; IBMX, 3-isobutyl 1-methylxanthine.

lowed us to avoid secondary neurogenic influences on the epithelium. Because we expected the rate of transport to be small and because we wished to learn how fluid composition was changed by epithelia, we measured changes that occurred over a period of 24 h. This approach also allowed us to ask how several mediators alter fluid transport and composition. We investigated the effects of cAMP-mediated agonists because a number of hormones, autocrine, and neurotransmitters increase cellular levels of cAMP and stimulate Cl^- secretion across airway epithelia (for review see reference 1). Moreover, cAMP-mediated Cl^- secretion is defective in CF airway epithelia (for review see reference 4). We also investigated the effects of amiloride, because amiloride-sensitive Na^+ channels at the apical surface mediate Na^+ absorption (measured as short-circuit current, I_{sc}). In addition, amiloride-sensitive Na^+ absorption is increased in CF airways (12), and has been the target of pharmacological intervention for the treatment of CF (13, 14).

Methods

Cell culture. Nasal polyps were obtained, after polypectomy, from 16 patients with allergic rhinitis. In addition, nasal turbinate specimens were obtained, after reconstructive surgery, from two patients. Airway epithelial cells were isolated by enzyme digestion as previously described (15–17). Freshly isolated cells were seeded at a density of 5×10^5 cells/cm² onto collagen-coated semipermeable membranes (24-mm diameter Transwell; Costar Corp., Cambridge, MA) and maintained at 37°C in an humidified atmosphere of 5% CO_2 in air. The culture media, a mixture of 50% DME and 50% Ham's F12 (DME/F12), was supplemented with 10 $\mu\text{g}/\text{ml}$ insulin, 5% fetal calf serum (Sigma Chemical Co., St. Louis, MO), 10 mM nonessential amino acids (Irvine Scientific, Santa Ana, CA), 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin.

Fluid transport. To assess the confluency of the monolayers, trans-epithelial resistance was measured with an ohmmeter (EVOM; World Precision Instruments, Sarasota, FL); we used monolayers with an electrical resistance $\geq 200 \Omega$ above the resistance measured across unseeded Transwell (4.5 cm²) membranes. To measure net fluid transport, we used techniques similar to those described by Mangoo-Karim et al. (18). The submucosal solution was replaced with 2.5 ml of fresh media (supplemented as described above); the mucosal solution was replaced with 100 μl of unsupplemented media. It was necessary to begin the experiment with some fluid on the mucosal surface because preliminary studies showed that if we began with no fluid on the mucosal surface, under most conditions, we could not recover sufficient fluid for analysis. Monolayers were exposed to one of the following conditions: control, no inhibitors or secretagogues; amiloride (30 μM) added to the mucosal solution; cAMP agonists, 10 μM forskolin and 100 μM 3-isobutyl 1-methylxanthine (IBMX) added to the submucosal solution; or amiloride plus cAMP agonists, final concentrations as indicated above. To minimize evaporative losses, the mucosal solution was covered with 1.5 ml of filter-sterilized mineral oil (previously warmed to 37°C, humidified, and equilibrated with 5% CO_2). Coating the mucosal solution with mineral oil did not alter the transport properties of epithelia. Monolayers covered with oil for 24 h were mounted in Ussing chambers: baseline I_{sc} remained amiloride-sensitive and cAMP-mediated secretagogues stimulated an increase in I_{sc} (not shown). The values were not different from monolayers not covered with oil.

After an incubation period of 24 h, the mucosal and submucosal solutions were collected for analysis. Mucosal solutions were collected in capillary tubes to measure the recovered volume using the length of the aqueous fluid column and the known internal diameter of the capillary tubes (custom glass tubing; Drummond Scientific Co., Broomall, PA).

Ion concentrations. Na^+ and K^+ concentrations were measured with a flame photometer (Instrumentation Laboratory, Inc., Boston,

MA); Cl^- concentrations were measured with a chloridometer (Laboratory Glass and Instruments Corp., New York). Neither Na^+ nor Cl^- concentrations were significantly modified by the addition of amiloride, forskolin and IBMX, or by supplementing the media with serum. However, K^+ concentrations in serum-supplemented media (4.46 ± 0.01 mM) were significantly greater than in unsupplemented media (4.04 ± 0.02 mM; $n = 20$).

After equilibration with 5% CO_2 , pH was measured with a micro pH electrode (model PHR-146; Lazar Research Labs, Los Angeles, CA). A standard pH curve was routinely obtained before measuring the pH of recovered solutions; standard solutions were simple electrolyte solutions to which a known quantity of NaHCO_3 had been added, and then equilibrated with 5% CO_2 . Over the pH range from 6.5 to 8.0, measurements were within 0.04 U of the predicted pH values (Henderson-Hasselbalch); in addition, values were identical whether pH was measured using micro or bulk volumes. HCO_3^- concentrations were calculated from the measured pH values using the Henderson-Hasselbalch equation.

To quantitate net H^+ transport, equivalent proton fluxes were determined by measuring the molar proton equivalents required to change the pH of the starting solution to the pH value of the recovered solution. While continuously gassed with a 5% CO_2 /95% O_2 mixture, 15 ml of media was titrated with 0.1 N HCl to the appropriate pH values. Proton fluxes were determined separately for serum-supplemented (submucosal) and unsupplemented (mucosal) media ($n = 3-6$); equivalent proton fluxes were adjusted for the volume of recovered fluid.

For each monolayer, the concentration of unmeasured anions (anion gap) was calculated as the sum of Na^+ and K^+ concentrations minus the Cl^- and HCO_3^- concentrations. On the basis of the manufacturer's insert, the concentrations of 21 amino acids, pyruvate, biphosphate, sulfate, nitrate, and trace additives accounted for over 90% of the anion gap of freshly prepared, unsupplemented media (16 mM); the media also contained 17.5 mM D-glucose.

Lactate concentrations were measured using an enzyme assay (735-UV; Sigma Chemical Co.). Lactate concentrations in unsupplemented and serum-supplemented media were 0.1 ± 0.1 and 1.1 ± 0.1 mM, respectively.

Data analysis. For each tissue specimen, one to six monolayers were used for each condition (control, amiloride, cAMP agonists, and amiloride plus cAMP agonists). When more than one monolayer was studied per condition, a single value (mean of two to six monolayers) was obtained for each condition. Thus each specimen contributed equally to the final means. All studies from an individual tissue specimen were carried out concurrently. Data are presented as mean $\pm$ SEM. Statistically significant differences between means were assessed using one- and two-way analysis of variance; P values ≤ 0.05 were considered statistically significant.

Reagents. Amiloride was a gift from Merck Sharp & Dohme (West Point, PA); SCH 28080 was obtained from Schering Corporation (Kenilworth, NJ). All other chemicals were purchased from Sigma Chemical Co.

Results

Fluid absorption and secretion. We first determined the volume of fluid that could be recovered from cultured epithelia. We replaced the mucosal solution of epithelial monolayers with 100 μl of media, and then coated it with mineral oil. 5–10 min later, $95.4 \pm 0.9 \mu\text{l}$ of the solution was recovered ($n = 20$); this value served as the predicted volume that should be recovered from monolayers that did not absorb or secrete fluid.

Fig. 1A shows the volume of mucosal fluid recovered from airway epithelia after the 24-h incubation period. The volume recovered from control monolayers was less than the 95 μl predicted for no net fluid transport. However, the volume recovered from amiloride-exposed monolayers was not different

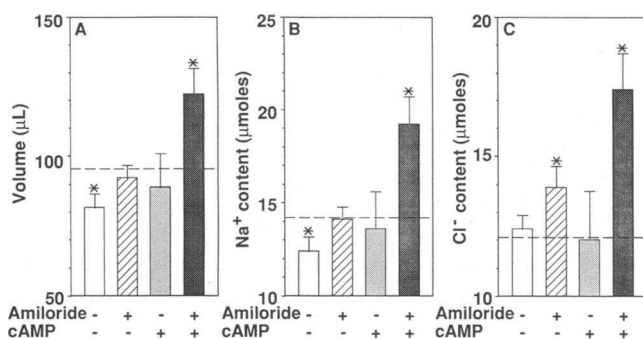


Figure 1. Effect of amiloride and cAMP on (A) volume, (B) Na⁺ content, and (C) Cl⁻ content of the mucosal solution recovered from cultured airway epithelia. The plus sign at the bottom of the figure indicates the presence of amiloride (30 μM) in the mucosal solution or cAMP agonists in the submucosal solution; cAMP represents the presence of forskolin (10 μM) and IBMX (100 μM) in the submucosal solution. The dashed line indicates the predicted volume or ion content recoverable from the mucosal surface (see Results for details); the predicted volume and Na⁺ and Cl⁻ contents were 95.4±0.9 μL, 14.2±0.2 μmol, and 12.1±0.1 μmol, respectively. Values are mean±SEM, *n* = 12; *Values that differ significantly from predicted values.

from the predicted value. This result indicates that, under basal conditions, airway epithelia absorb fluid. Because previous work (19, 20) has shown the presence of amiloride-sensitive, electrogenic Na⁺ absorption by canine and human airway epithelia, these results suggest that active Na⁺ absorption is responsible for the fluid absorption.

In contrast, addition of both amiloride and cAMP agonists (forskolin and IBMX) stimulated active fluid secretion (Fig. 1 A). This result is consistent with previous studies that have shown cAMP-mediated secretagogues stimulate Cl⁻ secretion across airway epithelia (21–24).

One surprising result was the apparent lack of fluid secretion in response to the addition of cAMP agonists alone (Fig. 1 A). However, examination of the results obtained from individual monolayers showed significant intersubject variability. Fig. 2 B shows that the response to cAMP was heterogeneous: cAMP agonists stimulated fluid secretion in some monolayers, whereas in others they stimulated fluid absorption. This variability is not explained by experimental error because multiple monolayers obtained from a single specimen always showed a consistent pattern of response. Furthermore, the response to amiloride or amiloride plus cAMP agonists was much more homogeneous: both treatment groups had a nearly uniform increase in mucosal fluid volume (Fig. 2, A and C).

Transepithelial transport of Na⁺ and Cl⁻. To gain insight into the mechanisms responsible for the fluid transport, we measured the Na⁺ content of the mucosal fluid (Na⁺ concentration × recovered volume). We compared this value with the Na⁺ content that would be recovered from the mucosal surface if there was no net fluid transport (Na⁺ concentration of the initial mucosal media × the predicted recoverable volume, 95.4 μL). The pattern of changes for each condition are the same as observed for the changes in fluid volume (compare Fig. 1, A and B). That is, for control monolayers, the content of Na⁺ on the mucosal surface decreased; amiloride prevented the decrease. The combination of amiloride plus cAMP agonists increased the mucosal Na⁺ content. These results suggest

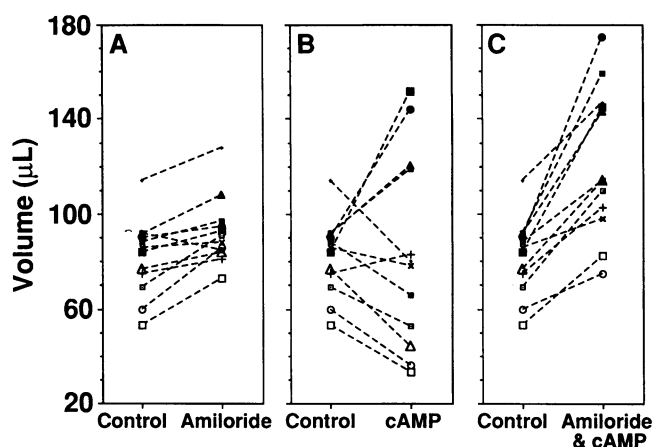


Figure 2. Effect of (A) amiloride, (B) cAMP agonists, and (C) amiloride plus cAMP agonists on the volume of recovered mucosal fluid. Each point represents data from a different specimen (the mean value of one to six monolayers). Dashed lines connect data points from the same specimen. Other details are as in Fig. 1.

that the distribution of fluid across airway epithelia parallels the distribution of Na⁺.

Interestingly, Cl⁻ transport differed in two ways from that of fluid and Na⁺ transport. First, mucosal Cl⁻ recovered from control monolayers was near the predicted value (Fig. 1 C). This observation suggests that under basal conditions Na⁺ absorption may be accompanied by anions other than Cl⁻ (discussed below). Second, mucosal Cl⁻ recovered from amiloride-treated monolayers was greater than predicted. One explanation for this result is that there is Cl⁻ conductance in the apical membrane under basal conditions and that addition of amiloride hyperpolarizes the apical membrane, thereby increasing the electrochemical gradient for Cl⁻ efflux. This possibility is consistent with the observations of Boucher and colleagues (12, 20). This explanation would still, however, require absorption of some other anion. The content of Cl⁻ on the mucosal surface was greatest for monolayers exposed to both amiloride and cAMP agonists: cAMP agonists would be expected to further increase apical Cl⁻ conductance.

Transepithelial Na⁺ and Cl⁻ concentration gradients. Fig. 3, A and B, shows the concentrations of Na⁺ and Cl⁻ in the mucosal and submucosal solutions. The concentrations of Na⁺ in the mucosal and submucosal solutions were not significantly different under basal conditions or in the presence of either amiloride or cAMP agonists added alone (Fig. 3 A). However, the mucosal Cl⁻ concentration exceeded the submucosal Cl⁻ concentration under basal conditions (Fig. 3 B). The Cl⁻ concentration gradient under control conditions could be accounted for by net Na⁺ and fluid absorption in the absence of net transepithelial Cl⁻ transport (Fig. 1, A–C); some anion other than Cl⁻ accompanied Na⁺ absorption. The mucosal Cl⁻ concentration also exceeded the submucosal concentration in the presence of amiloride (Fig. 3 B). As previously noted, this result is consistent with a basal degree of apical Cl⁻ conductance. A surprising finding, and one that is difficult to explain, is that cAMP agonists abolished the transepithelial Cl⁻ concentration gradient. This result may have been due to an insufficient driving force for Cl⁻ efflux through an apical Cl⁻ conductance under this condition. Alternatively, cAMP may have inhibited some Cl⁻/anion exchange process.

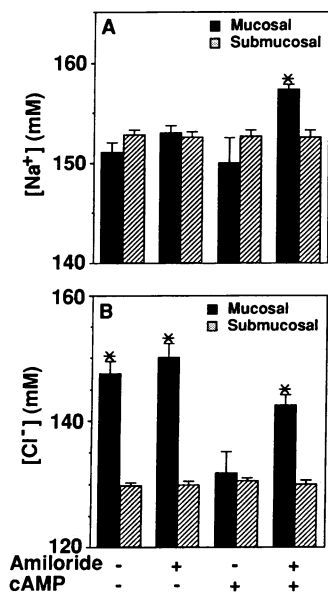


Figure 3. Effect of amiloride and cAMP agonists on the concentration of (A) Na^+ and (B) Cl^- in recovered mucosal and submucosal solutions. Mucosal and submucosal Na^+ concentrations at the beginning of the experiment were 148.8 ± 0.6 and 149.8 ± 0.5 mM, respectively; initial mucosal and submucosal Cl^- concentrations were 126.7 ± 0.7 and 126.8 ± 0.6 mM, respectively. *Mucosal concentrations that differ significantly from submucosal concentrations. Other details are as in Fig. 1.

When monolayers were exposed to the combination of amiloride plus cAMP agonists, the concentration of both Na^+ and Cl^- in the mucosal solution exceeded the concentration in the submucosal solution. This result is consistent with secretion of NaCl -rich fluid and passive water transport from submucosal to mucosal surface in response to an osmotic pressure difference.

Absorption of K^+ and secretion of H^+ . Although K^+ and H^+ concentrations are much lower than Na^+ and Cl^- concentrations, the transport of K^+ and H^+ across airway epithelia could alter the composition of the respiratory tract fluid. Under control conditions, the mucosal K^+ content decreased (Fig. 4 A); this result indicates that airway epithelia absorb K^+ under basal conditions. Mucosal amiloride had no effect on K^+ absorption. However, cAMP agonists inhibited net K^+ absorption.

The decrease in K^+ content in the apical compartment is the result of active transport. It cannot be explained by passive movement through paracellular pathways because the lumen-

negative transepithelial voltage and the transepithelial K^+ concentration difference at the start of the study (mucosal K^+ concentration 4.04 mM and submucosal K^+ concentration 4.46 mM, see Methods) would favor K^+ secretion rather than absorption. In addition, the predicted electrochemical gradient for K^+ across the apical membrane should favor K^+ efflux from the cell rather than K^+ influx (1). Thus an energy-dependent transport process appears to be responsible for absorption of K^+ by the epithelial cells.

The changes in K^+ content were paralleled by changes in the HCO_3^- content (Fig. 4 B) and pH (Table I). The decrease in pH indicates net H^+ secretion or, the equivalent, HCO_3^- absorption. We measured equivalent proton flux to quantitate net H^+ secretion. Net proton flux into the mucosal solution under basal conditions (Table I) was greater in magnitude than the decrease in HCO_3^- content (Fig. 4 B). This observation is consistent with the buffering effects of media and CO_2 . cAMP agonists inhibited net H^+ secretion (Table I) and the decrease in HCO_3^- content (Fig. 4 B). Amiloride had no effect, in either the presence or absence of cAMP agonists.

The presence of K^+ absorption and H^+ secretion, both of which were inhibited by cAMP agonists, suggested the possibility that K^+ absorption and H^+ secretion might be coupled. It seemed possible that an apical membrane K^+ pump, for example, H^+/K^+ -ATPase, might contribute to both results. Note, however, that it is unlikely that H^+/K^+ -ATPase would be the sole mechanism of net H^+ secretion because the magnitude of H^+ secretion (equivalent proton flux) significantly exceeded net K^+ absorption (Fig. 4 A).

To test whether K^+ was absorbed via H^+/K^+ -ATPase, we measured net transport across monolayers exposed to mucosal SCH 28080, a specific inhibitor of H^+/K^+ -ATPase in some epithelial cells (25–28). Fig. 5 shows that SCH 28080 inhibited net K^+ absorption. SCH 28080 had no effect on net fluid or Na^+ absorption (not shown). Net H^+ secretion was not inhibited by SCH 28080; this observation supports the notion that H^+ secretion is not mediated solely by SCH 28080-sensitive pathways (discussed below). These results are consistent with K^+ absorption via H^+/K^+ -ATPase. Nevertheless, SCH 28080 may have effects in addition to inhibition of H^+/K^+ -ATPase.

Table I. Effect of Amiloride and cAMP Agonists on pH and Net H^+ Flux

Condition	Mucosal		Submucosal	
	pH	H^+ flux μmol	pH	H^+ flux μmol
Control	$6.47 \pm 0.06^*$	0.81 ± 0.04	6.90 ± 0.02	12 ± 1.3
Amiloride	$6.41 \pm 0.06^*$	1.03 ± 0.05	6.93 ± 0.02	9 ± 1
cAMP	6.83 ± 0.05	$0.31 \pm 0.01^\ddagger$	6.86 ± 0.03	15 ± 1.5
Amiloride and cAMP	6.81 ± 0.05	$0.55 \pm 0.02^\ddagger$	6.88 ± 0.02	17 ± 1.5

H^+ flux represents the molar proton equivalents required to change the pH of the starting media to the pH values listed above (see Methods for details); flux determinations take into account the volume of recovered solution and whether the solution was supplemented with serum (submucosal solutions). * Mucosal pH values that differ significantly from submucosal pH values; ‡ Mucosal proton fluxes for cAMP-treated epithelia that differ significantly from the mucosal flux determinations for epithelia not treated with cAMP agonists.

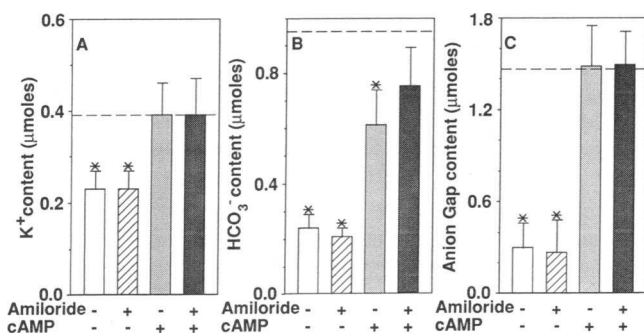


Figure 4. Effect of amiloride and cAMP agonists on the content of (A) K^+ , (B) HCO_3^- , and (C) anion gap in mucosal solution recovered from cultured airway epithelia. The dashed line indicates the predicted ion content recoverable from the mucosal surface (see Results for details); the predicted content for K^+ , HCO_3^- and anion gap were: 0.39 ± 0.01 , 0.97 ± 0.05 , and 1.5 ± 0.1 μmol , respectively. Other details are as in Fig. 1.

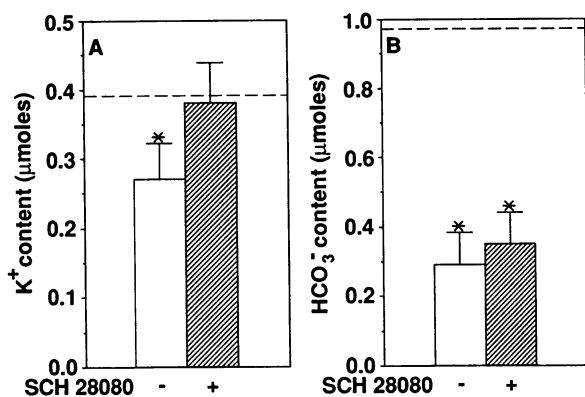


Figure 5. Effect of SCH 28080 on (A) K⁺ and (B) HCO₃⁻ content in the mucosal solution recovered from cultured airway epithelia. The plus sign at the bottom of the figure indicates the presence of SCH 28080 (30 μM). The dashed line indicates the predicted ion content recoverable from the mucosal surface (see Results for details); the predicted values for K⁺ and HCO₃⁻ contents were 0.39±0.01 and 0.97±0.05 μmol, respectively. Monolayers were not exposed to amiloride or cAMP agonists. Values are mean±SEM, *n* = 6. Other details are as in Fig. 1.

Transepithelial K⁺ and H⁺ concentration gradients. Fig. 6 A shows the K⁺ concentrations in the mucosal and submucosal solutions. The mucosal and submucosal K⁺ concentrations differed by ≤ 2 mM and presumably had minimal effects on net fluid transport.

We considered the mechanisms regulating acid–base balance by examining the pH and net H⁺ flux. Table I shows that epithelial monolayers acidified both mucosal and submucosal solutions. This result indicates that airway epithelia produced an organic acid during the 24-h study period. In addition, under basal conditions, epithelial monolayers generated a transepithelial H⁺ concentration gradient with the mucosal solution being more acidic than the submucosal solution. This pH gradient was accompanied by an increase in the anion gap of the submucosal solution (Fig. 6 C). The sizable anion gap is consistent with the submucosal accumulation of an anion other than Cl⁻ or HCO₃⁻. Taken together, the data are consistent with the net absorption of the sodium salt of an organic acid.

Lactic acid production. We considered that lactate, a by-product of glucose catabolism, might be responsible for some of the unmeasured anions in the recovered solutions and account for acidification of the media. Moreover, lactate production by native canine airway epithelia has been reported (29). Fig. 7 shows that lactate accumulated in both mucosal and submucosal solutions. Quantitatively, lactate production was greater than the total proton flux (Table I) and the change in anion gap (Fig. 6). However, these observations are consistent with the notion that airway epithelia also consume organic anions, e.g., amino acids and pyruvate. Although the concentrations of pyruvate and amino acids were not measured, the decrease in mucosal anion gap (Fig. 6 C) is also consistent with the consumption of organic anions. Lactate production was not due to coating the mucosal solution with mineral oil because lactate concentrations in media collected from epithelial monolayers coated with mineral oil (Fig. 7) were similar in magnitude to the lactate concentrations in media collected from uncoated airway monolayers (13±3.1 mM; *n* = 4).

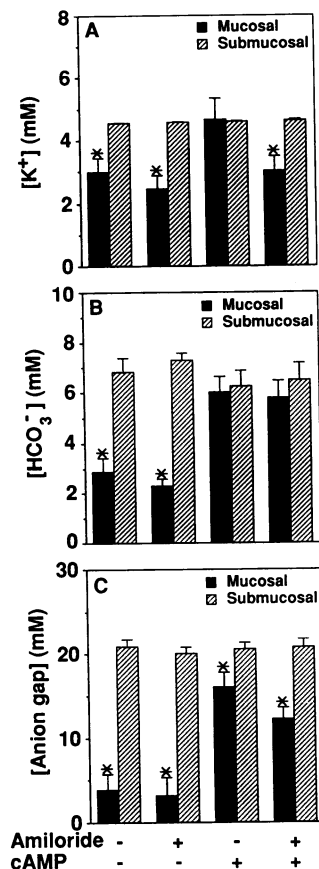


Figure 6. Effect of amiloride and cAMP agonists on the concentration of (A) K⁺, (B) HCO₃⁻, and (C) anion gap in the mucosal and submucosal solutions. Initial mucosal and submucosal K⁺ concentrations were 4.04±0.02 and 4.46±0.01 mM, respectively (see Methods); initial mucosal and submucosal HCO₃⁻ concentrations were 10.2±0.4 and 10.5±0.6 mM, respectively. Initial mucosal and submucosal anion gap concentrations were 15.8±0.7 and 16.9±0.9 mM, respectively. Values are mean±SEM, *n* = 12. Other details are as in Fig. 3.

Discussion

Understanding the fluid and electrolyte transport properties of airway epithelia requires knowledge of the rate of fluid transport, its regulation, and the ion composition of the surface fluid. This study provides the first such measurements in cultured human airway epithelia. Some of the results would be predicted by previous measurements of transepithelial ion fluxes; some were unexpected.

Fluid absorption by airway epithelium under basal conditions was consistent with predictions of previous studies (12, 30–32) that showed an absorptive radiotracer Na⁺ flux and an amiloride-sensitive *I*_{sc}. Our results and these previous studies indicate that active Na⁺ absorption drives fluid absorption. Because the rate of Na⁺ absorption by airway epithelia from patients with CF may be increased (12, 20), our results support the possibility that amiloride (or analogues thereof) may be of

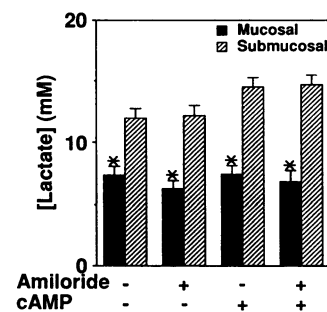


Figure 7. Effect of amiloride and cAMP agonists on lactate concentrations in recovered mucosal and submucosal solutions. Initial mucosal and submucosal lactate concentrations were 0.1±0.1 and 1.1±0.1 mM, respectively. Values are mean±SEM, *n* = 12. Other details are as in Fig. 3.

benefit in reducing fluid absorption across CF airway epithelia (13, 14), if a sufficient concentration of amiloride can be maintained at the airway luminal surface.

We found the Cl^- flux and concentration gradients surprising. Under basal conditions there was net Na^+ and fluid absorption, but there was no net transepithelial Cl^- transport (Fig. 1 C). This result suggests that some anion other than Cl^- accompanies Na^+ absorption. This interpretation is consistent with isotope flux measurements that indicate that net Na^+ absorption ($1.2 \pm 0.3 \mu\text{eq}/\text{cm}^2$ per h) across open-circuited human bronchial epithelium is not matched by Cl^- absorption ($0.5 \pm 0.3 \mu\text{eq}/\text{cm}^2$ per h) (33). Mechanistically, it is possible that Cl^- accompanies Na^+ absorption but that Cl^- absorption would then have to be counterbalanced by Cl^- secretion via a process that results in the net exchange of submucosal Cl^- for some other mucosal anion.

For monolayers treated with the combination of amiloride plus cAMP agonists, the epithelia secreted a NaCl-rich fluid. This result is consistent with previous reports (21, 31, 32) of transepithelial isotope fluxes and I_{sc} that indicate that cAMP stimulates Cl^- secretion by airway epithelia.

When added alone, cAMP agonists had no statistically significant effect on net transport. There was, however, significant intersubject variability (Figs. 1 and 2): cAMP stimulated net fluid secretion in some monolayers, whereas in others cAMP stimulated net fluid absorption. Our results do not identify the factor(s) that determine(s) whether an epithelium from one subject will absorb or secrete fluid when stimulated by cAMP. There are several previous observations that are relevant to this problem. First, cAMP agonists might directly stimulate Na^+ absorption; a cAMP-mediated increase in Na^+ absorption has been observed for canine tracheal epithelium bathed in Cl^- -free media (34) and for CF epithelia (12). In addition, there is strong evidence for cAMP-induced Na^+ absorption across alveolar epithelia (35, 36, 37) as well as epithelia of rat cortical collecting duct (38), amphibian distal nephron (A6) (39), turtle urinary bladder (40), and frog skin (41). Second, cAMP would be expected to increase apical Cl^- conductance (20, 42). However, the rate and direction of Cl^- movement will be determined by the electrical potential difference across the apical membrane. The apical voltage will in turn be regulated in large part by the apical Na^+ conductance (43, 44). Thus, we speculate that the magnitude of apical Na^+ conductance may play a part in modulating the effect of cAMP on fluid transport. This suggestion is consistent with our finding that, in the presence of amiloride, cAMP agonists stimulated secretion; by hyperpolarizing the apical membrane, amiloride would increase the driving force for Cl^- efflux.

Another unexpected finding was the absorption of K^+ by airway epithelia. This may have been unrecognized in previous studies because the magnitude of K^+ flux is much less than Na^+ and Cl^- fluxes. Moreover, K^+ absorption may be electrically neutral and thus undetectable in measurements of I_{sc} . Inhibition by SCH 28080 (Fig. 5) suggests that K^+ absorption occurs via an apical membrane H^+/K^+ -ATPase.

A transepithelial pH gradient was generated with a more acidic mucosal solution (Table I and Fig. 6). Interestingly, cAMP agonists inhibited both K^+ absorption and the generation of the pH gradient. This inhibitory effect of cAMP agonists is consistent with the notion that K^+ absorption and H^+ secretion may be coupled, for example, via H^+/K^+ -ATPase. However, differences in the magnitude of H^+ and K^+ flux (Fig. 4 A

and Table I) suggest an additional mechanism for net H^+ secretion, which is inhibited by cAMP agonists. We recently reported that cAMP stimulates an increase in apical HCO_3^- conductance in airway epithelia (45). Our results are consistent with the notion that the inhibitory effect of cAMP agonists on H^+ secretion may be due to a cAMP-induced increase in apical HCO_3^- conductance.

Cultured airway epithelia acidified the media; acidification was due in part to lactic acid production (Fig. 7). Because lactate concentrations were not equal in the mucosal and submucosal solutions, we considered physical factors that might account for the transepithelial lactate concentration gradients: a Donnan effect, the pH gradient, and the transepithelial voltage difference.

By attracting or repelling ions, charges on impermeable proteins may establish an ion gradient (Donnan effect). Using dialysis tubing that allows passage of molecules $< 3,500$ D, we observed lactate accumulation in media without serum (5.08 ± 0.02 mM) rather than serum-supplemented media (4.90 ± 0.04 mM; $n = 3$). Thus, the observed transepithelial lactate concentration gradients were not due to the presence of serum in the submucosal solution.

Using the Henderson-Hasselbalch equation, the pK_a for lactic acid (3.87) indicates that the concentration of lactic acid is much lower than lactate at pH 7 (46). In the presence of a transepithelial pH gradient, the ratio of lactate to lactic acid will differ for the mucosal and submucosal solutions. If the nonionic species (lactic acid) is membrane permeable and its concentration reaches an equilibrium across airway monolayers, then the measured transepithelial pH gradient under control conditions (submucosal pH 6.9 ± 0.02 vs. mucosal pH 6.47 ± 0.06) could account for a ratio of submucosal to mucosal lactate concentration of 2.7. This predicted ratio of lactate concentrations exceeds the measured ratio (1.6) of submucosal to mucosal lactate concentrations (Fig. 7). Therefore, the transepithelial pH gradients observed under control and amiloride-treated conditions could account for the lactate concentration gradients. However, transepithelial pH gradients were not present in cAMP-treated epithelia, thus another mechanism must account for the lactate concentration gradients in epithelia treated with cAMP agonists.

Using the Nernst equation, a lumen-negative transepithelial voltage of -12.7 mV could account solely for the observed transepithelial lactate concentration gradient (1.6) under basal conditions; a voltage of -19.7 mV could account solely for the concentration gradient (2.2) observed for epithelia treated with amiloride plus cAMP agonists. Although we did not measure the transepithelial voltages of these monolayers, values within this range have been reported for airway epithelia (1, 12, 20). Furthermore, although amiloride decreases and cAMP increases transepithelial voltage acutely (1, 12, 20), their effects on voltage after a period of 24 h are unknown. Thus, the combination of pH gradient and transepithelial voltage may account for the observed transepithelial lactate concentration gradients.

It is interesting to compare the electrolyte concentrations we report for mucosal fluid from cultured human airway epithelia, with previously reported values for airway surface fluid collected via a variety of techniques. Table II shows substantial variability in Na^+ and Cl^- concentrations in different species and airway regions as well as the presence of transepithelial concentration gradients. It is difficult to discern a specific pattern, but it is interesting that the Na^+ and Cl^- concentrations

Table II. Comparison of the Ion Concentrations in the Recovered Mucosal Solution under Basal Conditions with Previously Reported Concentrations in Airway Surface Fluid

Source	Na ⁺	Cl ⁻	K ⁺	HCO ₃ ⁻	Reference
Cultured human airway epithelia					
mucosal solution	151±0.9	148±2	3.0±0.4	2.8±0.6	—
Human trachea in vivo					
Nonsurgical case	93±4	91±5	11±1	—	11
Surgical case	91±12	108±15	19±3	low	
Canine airway in vivo					
Trachea	145±14	142±13.7	27±3.1	32±5.9	9
Bronchus	121±13	106±4.7	37±4.5	52±10	
Plasma	142±3	112±2.4	5.2±0.2	26±0.9	
Ferret airway in vitro					
Trachea	167±1.7	121±5.1	9.0±0.05	13*	54
Ringer's Solution	145	126	5.9	26	
Sheep lung					
Fetal lung liquid	150±1.3	157±4.1	6.3±0.7	2.8±0.3	50
Plasma	150±0.7	107±1.3	4.8±0.2	24±1.2	

* Value calculated from the measured pH using the Henderson-Hasselbalch equation.

we measured are higher than the hypoosmotic fluid recovered from human trachea (see Table II). A similarity between our results and most of the previous reports is the reduced mucosal HCO₃⁻ concentration. These results are consistent with net H⁺ secretion across airway epithelia. However, one consistent difference between our results and previous reports is the K⁺ concentrations: although K⁺ concentrations varied considerably in previous reports (6–37 mM), we found a lower concentration (3 mM). It is possible that the results of previous studies reflect the contributions of mucus, submucosal gland secretion, and intracellular ions (from injured epithelial cells or cells resident in the airway lumen). Alternatively, there may be substantial differences in the mechanisms of transepithelial K⁺ transport in different species or different regions of the airway. Finally, it is possible that cultured airway cells may not express a property of native epithelia (e.g., K⁺ secretion), although other properties appear to be retained in primary culture.

The pH of the recovered mucosal fluid ranged from 6.4 to 6.8. These values are within the range measured in vivo (47, 48) and the results are consistent with H⁺ secretion by airway epithelia. In fact, in vitro, ferret airway epithelia maintained airway surface fluid at an acidic pH (6.85±0.03) even when the submucosal solution was significantly alkalinized (49). Our observation of net H⁺ secretion and low HCO₃⁻ concentration is also consistent with the low pH of rabbit alveolar fluid (6.92±0.04) (50) and the low HCO₃⁻ concentration in fetal sheep lung liquid (51).

It is of interest to consider how the volume of fluid transported by cultured airway epithelia may affect the airways. The aqueous layer of airway surface fluid is estimated to be ~ 5 μm in depth in vivo (3); this corresponds to a volume of 500 nl of fluid over each cm² of epithelium. Thus, our measurements of net fluid transport under basal conditions correspond to the absorption of six times this volume of fluid over a period of 24 h. In contrast, for epithelia treated with amiloride and cAMP agonists, net fluid transport corresponds to the secretion of 12 times this volume over 24 h. This rate of fluid transport is less

than that measured across native canine tracheal (52) and human nasal epithelia (53); this difference may be due to culture conditions. Nevertheless, the rate of fluid transport by airway epithelia is within a range that is likely to impact on airway physiology. Furthermore, these results suggest that the inability to modify the rate of ion, and thus fluid transport across the airway, as occurs in CF epithelia, may significantly alter airway physiology.

In summary, these results provide new insights into the transport function of airway epithelia. An advantage of our techniques is that they isolate the transport properties to the surface airway epithelium; it will, however, also be interesting to learn what contribution submucosal gland secretions make to the composition of the respiratory tract fluid. In addition, it will be interesting to learn how abnormalities in ion transport by CF airway epithelia affect the composition of airway surface fluid. This technique may provide a useful adjunct for testing potential pharmacological and genetic therapeutic interventions for CF epithelia.

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