cAMP Stimulates Bicarbonate Secretion across Normal, but Not Cystic Fibrosis Airway Epithelia

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Abstract

Adenosine 3',5'-cyclic monophosphate stimulates chloride (Cl^-) secretion across airway epithelia. To determine whether cAMP also stimulates HCO_3^- secretion, we studied cultured canine and human airway epithelial cells bathed in a HCO_3^-/CO_2-buffered, Cl^--free solution. Addition of forskolin stimulated an increase in short-circuit current that was likely a result of bicarbonate secretion because it was inhibited by a HCO_3^--free solution, by addition of the carbonic anhydrase inhibitor, acetazolamide, or by mucosal addition of the anion channel blocker, diphenylamine 2-carboxylate. The current was dependent on Na^+ because it was inhibited by removal of Na^+ from the submucosal bathing solution, by addition of the Na^+ pump inhibitor, ouabain, or by addition of amiloride (1 mM) to the submucosal solution. An increase in cytosolic Ca^{2+} produced by addition of a Ca^{2+} ionophore also stimulated short-circuit current. These data suggest that cAMP and Ca^{2+} stimulate HCO_3^- secretion across airway epithelium, and suggest that HCO_3^- leaves the cell across the apical membrane via conductive pathways. These results may explain previous observations that the short-circuit current across airway epithelia was not entirely accounted for by the sum of Na^+ absorption and Cl^- secretion. The cAMP-induced secretory response was absent in cystic fibrosis (CF) airway epithelial cells, although Ca^{2+}-stimulated secretion was intact. This result suggests that HCO_3^- exit at the apical membrane is through the Cl^- channel that is defectively regulated in CF epithelia. These results suggest the possibility that a defect in HCO_3^- secretion may contribute to the pathophysiology of CF pulmonary disease. (*J. Clin. Invest.* 1992. 89:1148–1153.) Key words: pH • HCO_3^- transport • bicarbonate conductance

Introduction

Olver et al. (1) first reported that airway epithelia absorb sodium (Na^+) and secrete chloride (Cl^-). They observed that native canine tracheal epithelia bathed in Ringer's solution developed a lumen negative, transepithelial potential difference, suggesting that airway epithelia either absorb cations, secrete anions, or both. Under short-circuit conditions they measured a net flux of ^{36}Cl toward the airway lumen, and ^{22}Na toward the submucosa of tracheal epithelia. Their observation that airway epithelia absorb Na^+ and secrete Cl^- has been confirmed by numerous investigators studying nasal, tracheal, and bronchial epithelia from several species (for review see reference 2).

Since that initial report of transport across airway epithelium, the unidirectional flux of ^{22}Na and ^{36}Cl across native canine tracheal epithelia has been measured under short-circuit conditions by several investigators. Widdicombe et al. (3), and later Al-Bazzaz and Al-Awqati (4), found that Na^+ absorption and Cl^- secretion accounted for most of the short-circuit current (I_{sc})[1] although a small residual ion flux was present. Addition of dibutyryl cAMP stimulated an increase in both net Cl^- secretion and this residual current (5). When airway epithelia were bathed in Cl^--free solution, the net flux of Na^+ accounted for only 65% of the I_{sc} (4). In addition, when airway epithelia were exposed to furosemide, an inhibitor of basolateral Cl^- entry, Na^+ absorption and Cl^- secretion together accounted for only 55% of the I_{sc} (6).

These studies suggest that additional ion species may be transported across the airway. This notion was supported by our observation that cAMP-mediated agonists stimulated an increase in I_{sc} when airway monolayers were bathed in HCO_3^-/CO_2-buffered, Cl^--free solution, but not when both Cl^- and HCO_3^- were excluded from the bathing solution. Furthermore, this increase in I_{sc} was inhibited by diphenylamine 2-carboxylate (DPC), an anion channel blocker, but not by 10 μM amiloride, a Na^+ channel blocker. These findings led us to investigate whether airway epithelia secrete HCO_3^-, which might account for the unexplained residual ion flux noted in previous reports.

Methods

Cell culture. Airway cells were isolated from canine tracheal and human nasal epithelia using enzyme digestion, as previously described (7, 8). Nasal polyp specimens were obtained, after polypectomy, for upper airway obstruction in patients with allergic rhinitis (control) or cystic fibrosis (CF). Freshly isolated cells were seeded on collagen-coated, semipermeable membranes (Millicell-HA; Millipore, Bedford, MA), and incubated at 37°C in an humidified atmosphere of 5% CO_2 in air. Culture media, a mixture of 50% Dulbecco's modified Eagle medium and 50% Ham's F12 medium (DME/F12), was supplemented with 10 μg/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 μg/ml streptomycin.

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1. *Abbreviations used in this paper:* CF, cystic fibrosis; CPT- cAMP,8-(4-chlorophenylthio)-cAMP; DPC, diphenylamine 2-carboxylate; G_t, transepithelial conductance; IBMX, 3-Isobutyl 1-methylxanthine; I_{sc}, short-circuit current; NMDG, *N*-methyl D-glucamine.

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Table I. Composition of Bathing Solutions

No.	Na^+	$NMDG^+$	Cl^-	Gluconate	Aspartate	HCO_3^-	Hepes	SO_4^{-2}	CO_2
1	143	0	0	119	0	24	0	6.4	5%
2	0	119	0	119	0	24*	0	6.4	5%
3	119	0	0	119	0	0	10	6.4	0%
4	0	119	0	0	119	24	0	6.4	5%
5	143	0	0	0	119	24	0	6.4	5%
6	143	0	0	119	0	24	0	0‡	5%
7	119	0	119	0	0	0	10	6.4	0%
8	143	0	119	0	0	24	0	6.4	5%

Solutions contained (mM): $CaSO_4$ 2.4–3.2, $MgSO_4$ 1.2, K_2HPO_4 2.4, and KH_2PO_4 1.6 (pH = 7.4); * Choline bicarbonate substituted for $NaHCO_3$; ‡ Calcium acetate and $MgCO_3$ substituted for $CaSO_4$ and $MgSO_4$.

Short-circuit current measurements. Epithelial monolayers were mounted in modified Ussing chambers 4–7 d after seeding. They were bathed in symmetrical saline solutions (see Table I), warmed to 37°C, and gassed with a 95% O_2-5% CO_2 mixture (HCO_3^--free solutions were gassed with 100% O_2). The primary bathing solution was Cl^--free Ringer's, consisting of (mM) Na gluconate 119, $NaHCO_3$ 24, K_2HPO_4 2.4, KH_2PO_4 1.6, $CaSO_4$ 3.2, and $MgSO_4$ 1.2, pH = 7.4 (solution 1). For some experiments, this primary solution was modified using the ion substitutions listed in Table I.

Short-circuit current (I_{sc}) was recorded continuously; transepithelial conductance (G_t) was measured every 50 s by clamping to 5–20 mV. Experiments were performed with either KCl or Na gluconate agar bridges connecting Ussing chambers to Calomel electrodes; I_{sc} and G_t responses were not affected by the type of bridge used. For all recordings, amiloride (final concentration 10 μM) was present in the mucosal solution to inhibit Na^+ absorption. 3-Isobutyl 1-methylxanthine (IBMX) was added simultaneously with forskolin.

For some experiments, native canine tracheal epithelia were mounted in Ussing chambers and bathed in Cl^--free solution (solution 1); I_{sc} and G_t were recorded, as noted above.

Reagents. Amiloride was a gift from Merck, Sharp and Dohme Research (West Point, PA). Isoproterenol was obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ), and diphenylamine 2-carboxylate (DPC) from Fluka Chem. Corp. (Ronkonkoma, NY). All other chemicals were purchased from Sigma Chemical Co. All drugs were prepared as stock solutions (1,000-fold higher concentration); vehicle alone was added to control monolayers for comparison. Control and experimental studies were performed in paired monolayers from the same seeding.

Statistical methods. Values are presented as means±SEM. Statistically significant differences between means were measured using paired and unpaired t tests where appropriate; P values ≤ 0.05 were considered statistically significant.

Results

Bicarbonate secretion by airway epithelium. In Cl^--free solution (solution 1), canine airway monolayers had baseline electrical resistance and I_{sc} of 1000±190 $\Omega \cdot cm^2$, and 2.1±0.5 $\mu A/cm^2$, respectively (n = 16). Human airway epithelial monolayers had slightly lower values, but the values for normal and CF monolayers were similar: 440±50 and 460±80 $\Omega \cdot cm^2$, and 1.5±0.3 and 1.5±0.2 $\mu A/cm^2$ (n = 10), respectively. Addition of 10 μM amiloride to the mucosal solution decreased I_{sc} to 1.0±0.3 $\mu A/cm^2$ for canine cells, and to 0.4±0.2 and 0.2±0.1 $\mu A/cm^2$ for normal and CF nasal cells, respectively.

When forskolin, an activator of adenylate cyclase, was added to either the mucosal or submucosal solution, I_{sc} and G_t increased (Fig. 1). Several findings indicate that this response is mediated by an increase in intracellular cAMP concentration. First, addition of a membrane-permeable cAMP analogue, 8-(4-chlorophenylthio)adenosine 3',5'-monophosphate (CPT-cAMP), caused a similar increase in current (Fig. 2). Second, the increase in I_{sc} was greater in magnitude when forskolin or CPT-cAMP were added in the presence of IBMX, an inhibitor of cAMP catabolism (shown for CPT-cAMP). Third, isoproterenol and prostaglandin E_2, both secretagogues which increase intracellular cAMP concentration, also stimulated I_{sc} (shown for isoproterenol). Fourth, responses to each of these agonists were not additive, suggesting a common mechanism of action (shown for CPT-cAMP, forskolin and isoproterenol). These findings support the notion that the actions of these secretagogues are mediated by an increase in intracellular cAMP concentration.

To investigate the ionic basis of the cAMP-stimulated I_{sc}, we examined the effect of several agents that inhibit ion transport processes in airway epithelia. Mucosal addition of an anion channel blocker, DPC, inhibited the cAMP-mediated increase in I_{sc} (Fig. 1). G_t also decreased by 10% after DPC addition. These findings suggested that cAMP may stimulate HCO_3^- secretion via apical anion channels, although DPC might have effects other than anion channel blockade. As an alternative, we considered the possibility that cAMP may stimulate amiloride-insensitive Na^+ absorption.

We examined the effect of forskolin in monolayers that were bathed in either Na^+-free or HCO_3^--free solutions (solutions 2 and 3, respectively). In each case, the response to forskolin was attenuated (Fig. 3), indicating that both Na^+ and HCO_3^- were required for the increase in current.

Because HCO_3^--dependence might involve the conversion of CO_2 and H_2O to H^+ and HCO_3^-, we examined the effect of acetazolamide, an inhibitor of carbonic anhydrase, on the forskolin-stimulated response. Addition of acetazolamide caused an immediate decrease in I_{sc} (Fig. 4). In contrast, inhibitors of HCO_3^--coupled transport, 4,4'-diisothiocyanatostilbene-2,2'-disulfonate (200 μM DIDS), and 4-acetamido-4'-isothiocyanatostilbene 2,2'-disulfonate (250 μM SITS), did not attenuate the response to forskolin (n = 4 each).

These results suggested that the forskolin-stimulated current was due to HCO_3^- secretion, and that Na^+ might be

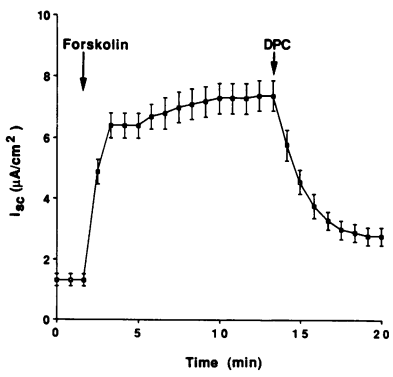

Figure 1. Effect of 10 μM forskolin on I_{sc} in canine epithelial monolayers bathed in Cl^--free solution (solution 1, see Table I). IBMX (100 μM) was added simultaneously with mucosal addition of forskolin (see Methods). Mucosal addition of 1 mM DPC inhibited the increase in I_{sc}. Forskolin increased G_t from 0.81±0.03 to 0.97±0.04 mS/cm²: G_t decreased to 0.89±0.04 mS/cm² after addition of DPC ($P < 0.05$). Values are means±SEM; n = 10.

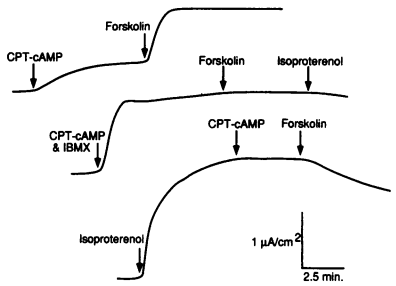

Figure 2. Effect of CPT-cAMP (50 μM) and isoproterenol (1 μM) on I_{sc}. Human airway monolayers were bathed in Cl^--free solution (solution 1). CPT-cAMP, forskolin (10 μM), and isoproterenol were added sequentially at the time indicated by arrows; 100 μM IBMX was added simultaneously with CPT-cAMP where indicated. Values are representative tracings from 3 monolayers.

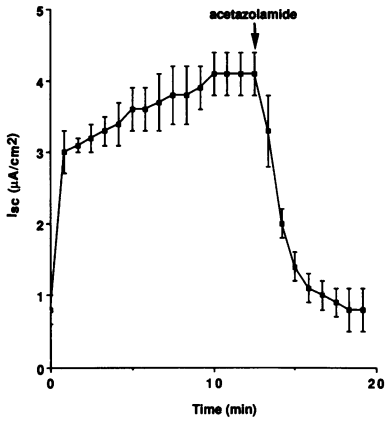

Figure 4. Effect of acetazolamide on forskolin-stimulated I_{sc}. Forskolin was added at time zero, acetazolamide (1 mM) was added to the submucosal solution at the time indicated by the arrow. Response to acetazolamide was identical whether added to mucosal or submucosal solution. Canine monolayers were bathed in solution 1. $n = 3$.

required for the process. To test this possibility, monolayers were bathed in symmetrical *N*-methyl D-glucamine aspartate solution (NMDG aspartate, solution 4). After 15–20 min in the Na^+-free solution, Na aspartate (solution 5) replaced either the mucosal or the submucosal NMDG aspartate. Establishing this transepithelial Na^+ concentration gradient resulted in a current in the direction expected for passive Na^+ permeability (Fig. 5). Subsequent addition of forskolin stimulated an increase in DPC-sensitive current in those monolayers exposed to Na^+ at the submucosal surface, but not those exposed to Na^+ at the mucosal surface. These results indicate that forskolin did not stimulate Na^+ absorption. Taken together with the HCO_3^- dependence (Fig. 3), these results suggest that forskolin stimulates Na^+-dependent, HCO_3^- secretion.

To further investigate the role of Na^+ in supporting HCO_3^- secretion, we examined the effect of Na^+ transport inhibitors on forskolin-stimulated I_{sc}. When monolayers were preincubated for 20–30 min with ouabain, an inhibitor of Na-K-ATPase activity, the response to forskolin was attenuated (Fig. 6). Ouabain attenuated this response only when added to the submucosal solution. This suggests that the transmembrane Na^+ gradient maintained by Na-K-ATPase is required for HCO_3^- secretion.

Na^+ might be required for HCO_3^- secretion in order to mediate the efflux of H^+ across the basolateral membrane (Na^+/H^+ exchange) when HCO_3^- exits across the apical membrane. To test this possibility, we examined the effect of 1 mM amiloride (For all experiments the mucosal solution contained 10 μM amiloride [see Methods]) on the forskolin-stimulated response; this high concentration of amiloride inhibits Na^+/H^+ exchange. Addition of 1 mM amiloride to the submucosal solution caused an immediate decrease in current (Fig. 7). In contrast, addition of 1 mM amiloride to the mucosal solution had no effect ($n = 4$). Moreover, addition of a lower concentration (10 μM) of amiloride to the submucosal solution had no effect. The inhibitory effects of amiloride and ouabain suggest that forskolin induces a recycling of Na^+ across the basolateral membrane via the Na^+/H^+ exchanger and Na-K-ATPase.

These results suggest that cAMP stimulates HCO_3^- secretion by the following mechanism: Carbonic anhydrase converts CO_2 and H_2O to H^+ and HCO_3^-. H^+ exits at the basolateral membrane and HCO_3^- exits across the apical membrane (For details, see Discussion). If HCO_3^- exits across the apical membrane through ion channels, the rate of transport should be influenced by the membrane potential. To determine whether membrane depolarization decreases the rate of HCO_3^- transport, we examined the effect of barium (Ba^{2+}), a potassium (K^+) channel blocker known to depolarize airway epithelia (9). Fig. 8 shows that Ba^{2+} caused an immediate inhibition of I_{sc}, supporting the notion that HCO_3^- crosses the apical membrane via a conductive pathway.

cAMP-stimulated HCO_3^- secretion was also observed in native airway epithelium mounted in Ussing chambers and bathed in HCO_3^-/CO_2-buffered, Cl^--free solution (solution 1). Forskolin stimulated an increase in I_{sc} (71±28 μA/cm², $n = 3$); secretion was blocked by mucosal addition of DPC (Fig. 9).

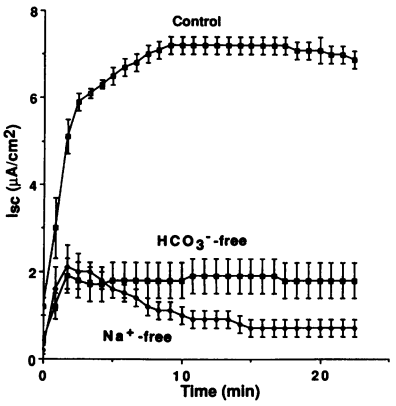

Figure 3. Effect of Na^+-free and HCO_3^--free solution on I_{sc} response to forskolin (canine monolayers). Control (solution 1), Na^+-free (solution 2), and HCO_3^--free (solution 3) solutions are Cl^--free (see Table I). Forskolin was added at time zero. Values are means±SEM; $n = 4$.

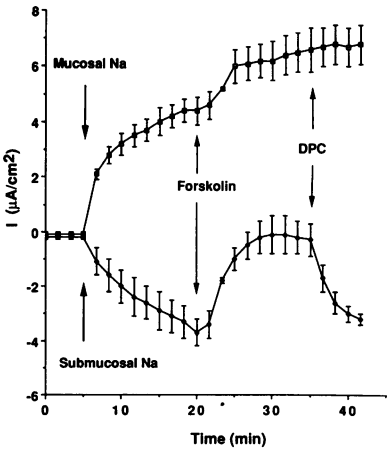

Figure 5. Effect of mucosal or submucosal Na^+ on forskolin-stimulated I_{sc}. Canine monolayers were bathed in symmetric NMDG aspartate solution (solution 4); Na aspartate (solution 5) replaced NMDG asparate in either the mucosal or submucosal solution (*first arrow*). Establishing this Na^+ concentration gradient resulted in a current in the direction expected for a passive Na^+ permeability. Forskolin and DPC were added at the times indicated by arrows ($n = 3$).

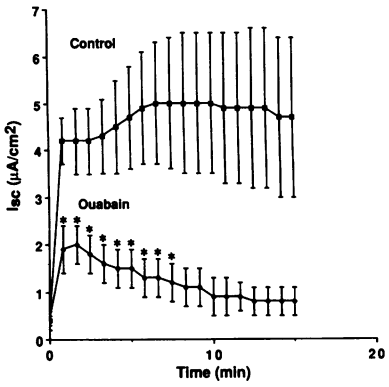

Figure 6. Effect of ouabain (10 μM) on forskolin-stimulated I_{sc}. Canine monolayers were bathed in solution 1. *Increase in I_{sc} significantly different from control. n = 3.

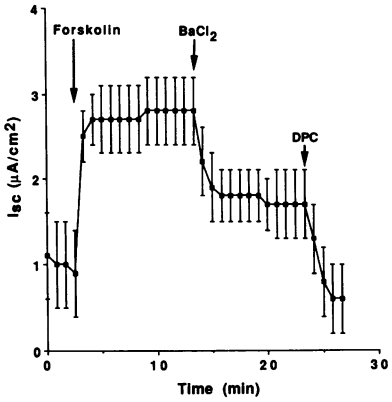

Figure 8. Effect of Ba^{2+} on forskolin-stimulated I_{sc}. Human airway monolayers were bathed in solution 6; forskolin (10 μM), $BaCl_2$ (1 mM) and DPC (1 mM) were added at the times indicated by arrows. $BaCl_2$ was added to both submucosal and mucosal solutions. n = 3.

Bicarbonate and chloride secretion. Because cAMP also stimulates Cl^- secretion by airway epithelia (2), we asked whether HCO_3^- secretion accounts for part of the increase in I_{sc} when epithelia are bathed in solution containing both Cl^- and HCO_3^-. If secretion of Cl^- and HCO_3^- are independent, we would expect the secretory responses to be additive. Fig. 10 shows that the response to forskolin was similar when monolayers were bathed in solutions containing either Cl^-, HCO_3^-, or both. These results suggest that cAMP-mediated Cl^- and HCO_3^- secretion are not independent. Furthermore, because the I_{sc} responses were not additive, Cl^- and HCO_3^- are unlikely to be secreted by different cell populations.

To further determine the relative contribution of Cl^- secretion and HCO_3^- secretion to the cAMP-induced increase in I_{sc}, we examined the effects of bumetanide and amiloride (1 mM) that inhibit Cl^- (10) and HCO_3^- (Fig. 7) secretion, respectively. Fig. 11 shows that bumetanide inhibited 81±2% of the forskolin-stimulated I_{sc} when monolayers were bathed in CO_2-free, NaCl solution (solution 7), and inhibited only 2±2% of the current when monolayers were bathed in HCO_3^-/CO_2-buffered, Cl^--free solution (solution 1). These results indicate that bumetanide inhibits Cl^- secretion, but not HCO_3^- secretion. When monolayers are bathed in solution containing both Cl^- and HCO_3^-, bumetanide inhibited only 33±7% of the I_{sc}; amiloride inhibited the remaining current (Fig. 11).

These results suggest that when epithelia are bathed in solution containing both anions, cAMP stimulates secretion of both Cl^- and HCO_3^-. However, the addition of bumetanide may have shifted current, due predominantly to Cl^- secretion to HCO_3^- secretion alone. The following results support this possibility. In solutions containing both Cl^- and HCO_3^- (solution 8), the addition of acetazolamide did not inhibit forskolin-stimulated I_{sc} (n = 7, also reference 20). However, when acetazolamide addition was preceded by bumetanide, which decreased I_{sc} by 33±1%, the subsequent addition of acetazolamide inhibited the remaining current (n = 8). These results suggest that HCO_3^- secretion may be low in the presence of Cl^-; HCO_3^- secretion may increase after Cl^- secretion is blocked by bumetanide. These studies do not allow us to determine, in cultured or native epithelia, the absolute amount of HCO_3^- secretion that occurs in the presence of Cl^-.

Defective bicarbonate secretion by CF tracheal epithelia. Our results suggest that HCO_3^- secretion occurs via a cAMP-stimulated conductive pathway in the apical membrane. If that were the case, we expected that HCO_3^- secretion would be defective in CF airway epithelia. To determine whether cAMP stimulates HCO_3^- secretion across CF nasal airway epithelia, control (atopic) and CF nasal epithelial monolayers were bathed in Cl^--free solution (solution 1); addition of forskolin stimulated an increase in I_{sc} in control, but not CF monolayers (Fig. 12). Although cAMP-mediated Cl^- secretion is defective in CF airway epithelia, Ca^{2+}-dependent Cl^- secretion is intact (11). Thus, we examined whether agonists that increase $[Ca^{2+}]_c$ stimulated HCO_3^- secretion. Bradykinin, ATP, or a calcium ionophore (A23187) were used to stimulate HCO_3^- secretion because these secretagogues increase $[Ca^{2+}]_c$ and stimulate Cl^- secretion across normal and CF airway epithelia (12, 13, 14, 15). In Cl^--free solution, each of these secretagogues alone stimulated a transient increase in I_{sc} with both normal and CF epithelia (Figs. 12 and 13).

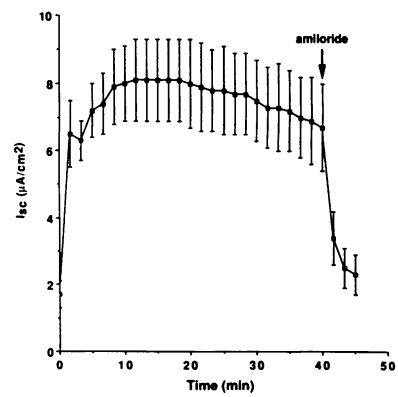

Figure 7. Effect of submucosal addition of amiloride on forskolin-stimulated I_{sc}. Forskolin was added at time zero, amiloride (1 mM) was added at time indicated by the arrow. Canine monolayers were bathed in solution 1. n = 3.

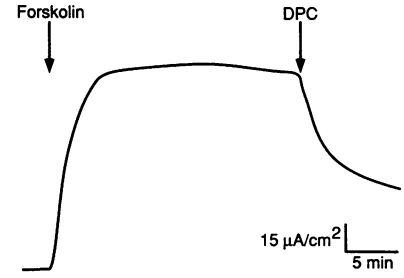

Figure 9. Effect of forskolin on I_{sc} across native canine tracheal epithelia mounted in HCO_3^-/CO_2-buffered, Cl^--free solution (solution 1). Representative tracing of I_{sc} in response to forskolin and DPC (0.5 mM) added to the mucosal solution at the time indicated by arrows; amiloride (10 μM) and indomethacin (1 μM) were added to the mucosal solution 20 min before addition of forskolin. G_t decreased from 2.1 to 1.6 mS/cm² after addition of DPC.

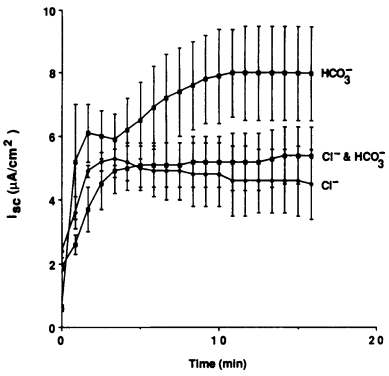

Figure 10. Effect of Cl^- and HCO_3^- on forskolin-stimulated I_{sc}. Bathing solutions contained either HCO_3^- (solution 1), Cl^- (solution 7), or both (solution 8), as indicated. Forskolin was added at time zero. Mean I_{sc} values between each group were not significantly different; n = 3 canine monolayers for each condition.

Discussion

Our results indicate that cAMP stimulates HCO_3^- secretion across airway epithelial cells; the results and our interpretation are summarized by the model in Fig. 14. In cultured airway cells, carbonic anhydrase converts H_2O and CO_2 to H^+ and HCO_3^-. H^+ exits across the basolateral membrane via Na^+/H^+ exchange; HCO_3^- exits across the apical membrane through anion channels. Basolateral Na-K-ATPase maintains a chemical driving force for H^+ efflux (the transmembrane Na^+ gradient); basolateral K^+ channels maintain an electrical driving force for HCO_3^- efflux.

The evidence that cAMP stimulates HCO_3^- transport through apical membrane anion channels is: (*a*) cAMP stimulates an increase in electrically conductive ion transport, i.e., I_{sc} and G_t increase; (*b*) both I_{sc} and G_t decrease after addition of the channel blocker, DPC; and (*c*) addition of Ba^{2+}, which inhibits basolateral K^+ channels and depolarizes airway epithelial cells, inhibited I_{sc}. Perhaps the most striking feature in this regard is the failure of cAMP to stimulate HCO_3^- secretion in CF epithelial cells. Although cAMP-stimulated secretion was deficient, Ca^{2+}-dependent stimulation was intact. These observations parallel previous observations on Cl^- secretion: cAMP-dependent Cl^- secretion is deficient whereas Ca^{2+}-stimulated Cl^- secretion is normal in CF airway epithelia (16). Because the apical membrane of CF airway epithelia display defective regulation of apical Cl^- channels, and because the protein mutated in CF, cystic fibrosis transmembrane conductance regulator (CFTR), is itself a cAMP-regulated Cl^- channel (17, 18, 19), our data suggest that HCO_3^- exit occurs through the same cAMP-regulated Cl^- channel that is defective in CF.

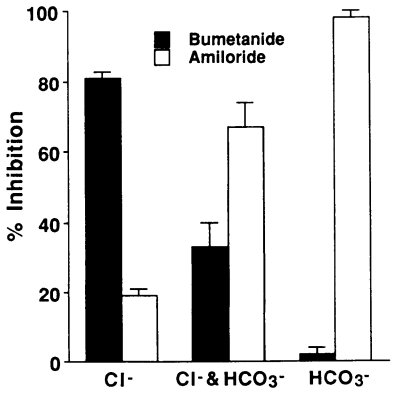

Figure 11. Effect of bumetanide and amiloride on forskolin-stimulated I_{sc}. Canine monolayers were bathed in solution containing either HCO_3^- (solution 1), Cl^- (solution 7), or both (solution 8), as indicated; forskolin (10 μM) was added to stimulate an increase in I_{sc}. Bumetanide (100 μM) and amiloride (1 mM) were added sequentially to the submucosal solution. Percent inhibition of I_{sc} bumetanide and amiloride is depicted; values are means±SEM (n = 4).

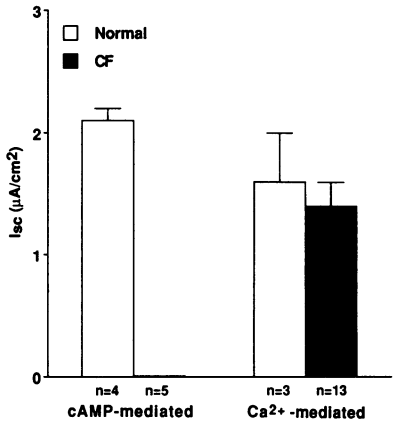

Figure 12. Effect of cAMP- and Ca^{2+}-mediated agonists on change in I_{sc} in normal and CF airway epithelia bathed in Cl^--free solution (solution 1). Forskolin (10 μM) was added for cAMP-mediated stimulation of I_{sc}; either bradykinin (1 μM), ATP (250 μM), or A23187 (10 μM) were added for Ca^{2+}-mediated stimulation of I_{sc}. n refers to the number of monolayers studied in each group.

Our results indicate that HCO_3^- secretion by airway epithelia is Na^+-dependent (Fig. 3). The notion that Na^+ is recycled across the basolateral membrane via Na-K-ATPase and the Na^+/H^+ exchanger is supported by the observations that Na^+ is required in the submucosal solution only (Fig. 5), and that secretion is inhibited by submucosal addition of ouabain or 1 mM amiloride (Figs. 6 and 7). Moreover, inhibition of Na^+/H^+ exchange by 1 mM amiloride is consistent with acetazolamide-inhibited HCO_3^- secretion (Fig. 4), which should involve a mechanism of H^+ efflux to maintain intracellular pH.

With the magnitude of HCO_3^- secretion across airway epithelia possibly as great as Cl^- secretion (Figs. 9 and 10), one might wonder why HCO_3^- secretion was not apparent in previous studies. The results from two studies suggested at first glance that HCO_3^- secretion was unlikely. First, Al-Bazzaz and Al-Awqati (4) reported that the removal of HCO_3^- from the bath did not diminish cAMP-stimulated I_{sc}. Our results are identical (Fig. 10), but they are also consistent with a shift from HCO_3^- and Cl^- secretion to that of Cl^- secretion alone (Figs. 10 and 11). Second, Widdicombe (20) observed that when monolayers were bathed in solutions containing Cl^- and HCO_3^-, the addition of acetazolamide did not attenuate the I_{sc} response to cAMP. We also observed this lack of inhibition by acetazolamide alone. However, after inhibiting Cl^- secretion with bumetanide, the subsequent addition of acetazolamide inhibited I_{sc}. These results are also consistent with the notion that the

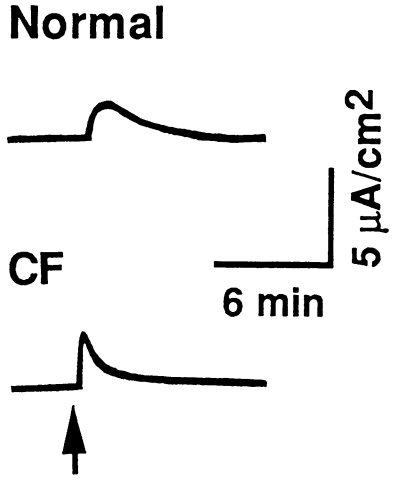

Figure 13. Effect of A23187 on I_{sc} in normal and CF airway monolayers. Monolayers were bathed in Cl^--free solution (solution 1); A23187 (1 μM) was added to the mucosal solution at the time indicated by the arrow; IBMX was not added. Values are tracings from representative monolayers.

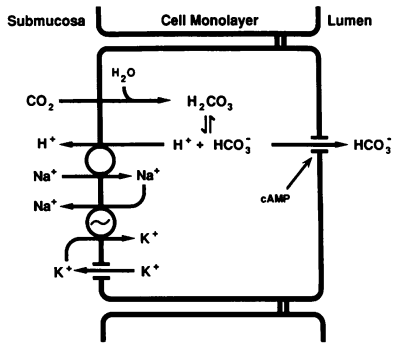

Figure 14. Model of cAMP-mediated, HCO_3^- secretion in cultured airway epithelium. Pathways involved in HCO_3^- transport are shown; other transporters are omitted for clarity. For details see text.

addition of acetazolamide may shift Cl^- and HCO_3^- secretion to Cl^- secretion alone. Another reason that HCO_3^- secretion might not have been previously identified is that Cl^--free solutions were also CO_2-free (buffered with Hepes rather than HCO_3^-/CO_2) (21). Finally, although initial studies suggested that airway epithelia do not secrete HCO_3^-, the simultaneous measurement of I_{sc} and unidirectional ion flux across airway epithelia did indicate a cAMP-stimulated, residual ion flux that was heretofore unexplained (3–6). Our results suggest that HCO_3^- secretion may have accounted for this puzzling observation.

What is the functional significance of HCO_3^- secretion by airway epithelia? Is the rate of cAMP-stimulated HCO_3^- secretion sufficient to alkalinize airway fluid? Although experimental data indicating alkalinization of the respiratory tract fluid has not been obtained, several factors support the possibility that such a process might be physiologically significant. First, the rate of HCO_3^- secretion by native epithelium can be quite high (over 100 $\mu A/cm^2$ in Cl^--free solution under short-circuit conditions). Although these conditions differ from those which occur in vivo, the capacity for HCO_3^- secretion is substantial. Second, the volume of fluid into which HCO_3^- would be secreted (the sol layer of airway secretions) is quite small, relative to the secretory surface area. The sol layer, which bathes airway cilia, is $\sim$ 5 μm in depth, and is covered by a mucus or gel layer (22). This low ratio of airway fluid volume to secretory surface area, and the potentially high rate of HCO_3^- secretion, would favor significant changes in pH following stimulation with secretagogues. Third, reports of HCO_3^- concentrations of 50 meq/ml (23) and pH values of 7.8–8.0 (24) in canine airway liquid are consistent with HCO_3^- secretion into the airway lumen; pH values of human airway liquid (patients with tracheostomy) ranged from 6.1 to 7.9 (25). The alkalinization of airway fluid might be important to pulmonary defense mechanisms such as mucociliary clearance; for example, ciliary beat frequency is optimal at pH values of 7.0 to 9.0 (26, 27). These results suggest the possibility that a defect in HCO_3^- secretion by airway epithelia may contribute to the pathophysiology of CF pulmonary disease.

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