JOP. J. Pancreas (Online) 2001; 2(4 Suppl):291-293.

cAMP Stimulation of HCO_3^- Secretion Across Airway Epithelia

Michael J Welsh, Jeffrey J Smith

Howard Hughes Medical Institute and Departments of Internal Medicine and Pediatrics, University of Iowa College of Medicine. Iowa City, IA, USA

Summary

To test for the presence of HCO_3^- transport across airway epithelia, we measured short-circuit current in primary cultures of canine and human airway epithelia bathed in a Cl^--free, HCO_3^-/CO_2-buffered solution. cAMP agonists stimulated a secretory current that was likely carried by HCO_3^- because it was absent in HCO_3^--free solutions. In addition, the cAMP-stimulated current was inhibited by the carbonic anhydrase inhibitor, acetazolamide, and by the apical addition of a blocker of cystic fibrosis transmembrane conductance regulator (CFTR), diphenylamine-2-carboxylate. The current was dependent on Na^+ because it was inhibited by removing Na^+ from the submucosal solution and by inhibition of the Na^+-K^+-ATPase with ouabain. The cAMP-stimulated current was absent in cystic fibrosis (CF) airway epithelia. These data suggest that cAMP agonists can stimulate HCO_3^- secretion across airway epithelia and that CFTR may provide a conductive pathway for HCO_3^- movement across the apical membrane.

For many years it has been known that under short-circuit conditions that airway epithelia absorb Na^+ and secrete Cl^-. However, several studies have suggested that the unidirectional fluxes of Na^+ and Cl^- did not entirely account for the short-circuit current (I_{sc}). This discrepancy has been observed in older studies using either native or cultured canine tracheal epithelia. These results suggested that additional ion species might be transported across the airways. Therefore, we tested the hypothesis that airway epithelia might secrete HCO_3^-.

For these studies we used primary cultures of canine tracheal epithelia; canine epithelia tend to have a larger cAMP-induced increase in CFTR-dependent anion transport than human airway epithelia. For these studies we used Cl^--free bathing solution in which gluconate replaced the bulk of Cl^-. HCO_3^- (24 mM) was included in the solution which was bubbled with 5% CO_2. To inhibit electrically conductive Na^+ transport, amiloride (10 µM) was present in the mucosal solution for all studies.

We initially evaluated the effect of cAMP agonists on I_{sc}. Figure 1 shows the results. Transepithelial current increased following addition of forskolin (10 µM). We obtained similar results with addition of other agonists that increase cellular levels of cAMP such as superterenol, prostaglandin E2, and CTP-cAMP. These findings suggested that cAMP might stimulate bicarbonate secretions via apical anion channels.

To investigate the ionic basis of the cAMP-stimulated I_{sc}, we examined the effect of ion

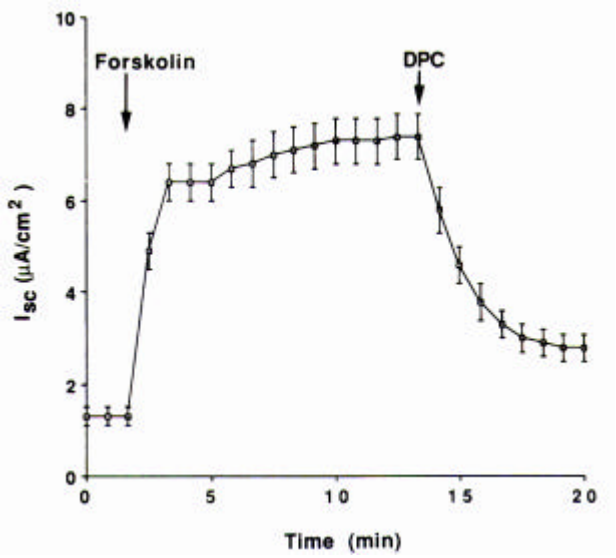

Figure 1. Effect of Forskolin and DPC on I_{sc} in cultured canine airway epithelia bathed in Cl^--free Ringers solution. From reference [1].

substitutions and transporter inhibitors. Addition of diphenylamine 2-carboxylate (DPC) (Figure 1) inhibited the majority of the current. In either Na^+-free or HCO_3^--free solutions, the effect of forskolin was markedly attenuated, indicating that both Na^+ and HCO_3^- are 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. Addition of acetazolamide caused an immediate decrease in I_{sc}. In contrast, inhibitors of HCO_3^--coupled transport (200 μM 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS) or 250 μM 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS) did not inhibit the response to forskolin.

In additional studies we found that forskolin stimulated HCO_3^- secretion required Na^+ on the basolateral surface. These results suggest that forskolin stimulates a Na^+-dependent HCO_3^- secretion. We also found that HCO_3^- secretion was inhibited by oubain, which inhibits the Na^+-K^+-ATPase. Likewise, addition of 1 mM amiloride to the basolateral surface, to inhibit Na^+-proton exchangers, or addition of Ba^{2+}, a K^+ channel blocker known to depolarize airway epithelia, inhibited transport.

To test the dependence of HCO_3^- transport on CFTR, we compared non-CF and CF airway epithelia. Figure 2 shows that following the inhibition of Na^+ transport by amiloride the addition of forskolin and 3-isobutyl 1-methylxanthine (IBMX) stimulated a small secretory current in the Cl^--free solutions. Subsequent addition of acetazolamide inhibited current. These data suggest that the electrically conductive HCO_3^- secretion in airway epithelia involves HCO_3^- flow through the CFTR Cl^- channel.

In subsequent studies 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^-. We used a series of ion substitution studies, addition of bumetanide, which inhibits Cl^- entry at the basolateral membrane, and acetazolamide. The data suggested that when epithelia were bathed in solutions containing both anions, cAMP stimulated secretion of both Cl^- and HCO_3^-. However, the data did not allow us to determine the absolute amount of HCO_3^- secretion that occurs in the presence of Cl^-.

Our results indicate that cAMP stimulates HCO_3^- secretion across canine and human airway epithelial cells. Our interpretation of the results are summarized in Figure 3. In airway epithelia, carbonic anhydrase converts H_2O and CO_2 to H^+

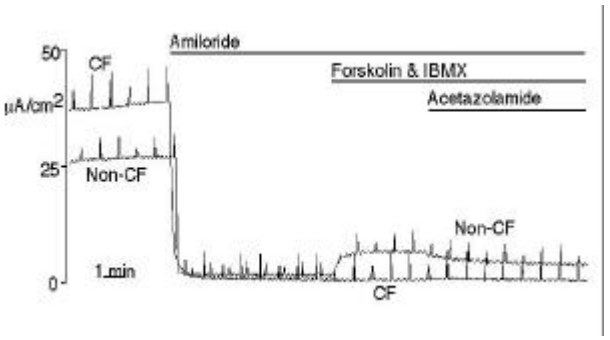

Figure 2. Effect of cAMP agonists and acetazolamide on I_{sc} in primary cultures of well-differentiated human airway epithelia bathed in Cl^--free Ringers solution.

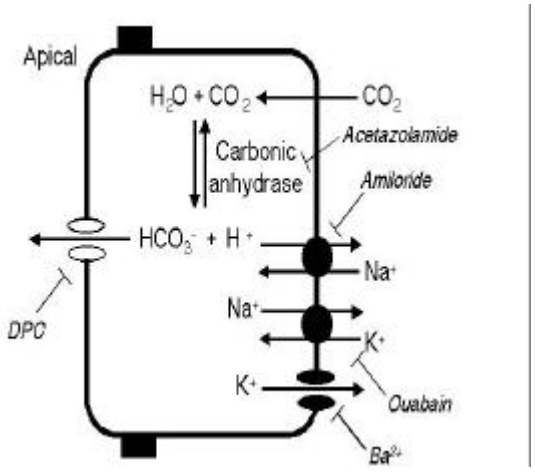

Figure 3. Model of HCO_3^- secretion across airway epithelia under short-circuit conditions in Cl^--free solutions. See text for details.

and HCO_3^-. H^+ exits across the basolateral membrane via a Na^+/H exchanger. HCO_3^- exits across the apical membrane through CFTR. The basolateral Na^+-K^+-ATPase maintains a chemical driving force for H^+ efflux (i.e., the transmembrane Na^+ gradient), and basolateral K^+ channels maintain an electrical driving force for HCO_3^- efflux. This process is stimulated by cAMP agonists.

What is the functional significance of HCO_3^- secretion by airway epithelia? Our experiments do not answer this question. The finding that there is an appreciable rate of HCO_3^- secretion, at least under short-circuit conditions, suggest that this might be sufficient to modify the pH in the very thin layer of airway surface liquid. If that is the case these results suggest the possibility that a defect in HCO_3^- secretion by CF airway epithelia might contribute to the pathophysiology of the disease.

Key words Bicarbonates; Cystic Fibrosis; Epithelium; Lung

Abbreviations CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; DPC: diphenylamine 2-carboxylate; IBMX: 3-isobutyl 1-methylxanthine; I_{sc}: short-circuit current; SITS: 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid

Acknowledgements This work was supported by the National Heart, Lung and Blood Institute and the Cystic Fibrosis Foundation.

Correspondence
Michael J Welsh
Howard Hughes Medical Institute
University of Iowa College of Medicine
500 EMRB, Iowa City, IA 52242
USA
Phone: +1-319.335.7619
Fax: +1-319.335.7623
E-mail address: mjwelsh@blue.weeg.uiowa.edu

References

1. Smith JJ, Welsh MJ. cAMP stimulates bicarbonate secretion across normal, but not cystic fibrosis airway epithelia. J Clin Invest 1992; 89:1148-53. [92210710]