Mechanisms of Bicarbonate Secretion: Lessons from the Airways

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Early studies showed that airway cells secrete HCO$_3^-$ in response to cAMP-mediated agonists and HCO$_3^-$ secretion was impaired in cystic fibrosis (CF). Studies with Calu-3 cells, an airway serous model with high expression of CFTR, also show the secretion of HCO$_3^-$ when cells are stimulated with cAMP-mediated agonists. Activation of basolateral membrane hIK-1 K$^+$ channels inhibits HCO$_3^-$ secretion and stimulates Cl$^-$ secretion. CFTR mediates the exit of both HCO$_3^-$ and Cl$^-$ across the apical membrane. Entry of HCO$_3^-$ on a basolateral membrane NBC or Cl$^-$ on the NKCC determines which anion is secreted. Switching between these two secreted anions is determined by the activity of hIK-1 K$^+$ channels.

The recognition that HCO$_3^-$ secretion is impaired in CF patients dates back to the studies of Hadorn and coworkers in the 1960s (Hadorn et al. 1968). These investigators showed that pancreatic HCO$_3^-$ and fluid secretion were diminished in CF patients. Moreover, CF patients were refractory to secretin, a cAMP-mediated secretory agonist in the pancreas. These studies were confirmed by Gaskin et al. (1982) and Kopelman et al. (1985, 1988). Extensive transport studies of the exocrine pancreas have shown that HCO$_3^-$ is the primary anion secreted by the ductal cells, the predominate site of CFTR expression (Marino et al. 1991). The human pancreas can secrete a fluid of 130 mM HCO$_3^-$ (Schultz 1987). Secreted HCO$_3^-$ electrically draws Na$^+$ into the lumen and H$_2$O follows osmotically. The secreted fluid and electrolytes serve to flush the digestive enzymes from the acini and ducts of the pancreas. Thus, impaired HCO$_3^-$ secretion results in poor clearance of the digestive enzymes, and their premature activation eventuates in the destruction of the pancreas in CF. We surmise that a similar sequela follows from impaired HCO$_3^-$ secretion in the submucosal glands and airways of CF patients. Indeed, several recent studies from Wine and coworkers have shown cAMP-stimulated fluid secretion is impaired from CFTR-deficient submucosal glands (Joo et al. 2006). Verkman and coworkers have also shown impaired fluid secretion from the submucosal glands of CF patients and shown that the secreted fluid is hyperviscous and acidic compared with glands from non-CF patients (Salinas et al. 2005; Song et al. 2006). Analogous to the pancreas, the submucosal glands secrete mucins, protease inhibitors, antibiotic peptides,
and enzymes that must be flushed from the glands onto the airway surface epithelium (Basaum et al. 1990). Moreover, the physical properties of mucus are intrinsically dependent on the composition of the fluid. Most notably, alterations in ionic strength, divalent cation concentration, and pH have profound effects on the viscoelastic properties of mucins (Forstner et al. 1976; List et al. 1978; Tam et al. 1981; Lin et al. 1993). In the pancreas, the pH of the ductal fluid plays a critical role in regulating the activity of the exocytosed digestive enzymes. In contrast, very little is known regarding the electrolyte composition and pH of the submucosal gland fluid and the role it might play in the 1000-fold expansion that a mucin granule undergoes upon release and degranulation (Yeates et al. 1997; Verdugo and Hauser 2012). In addition, the surface epithelium must maintain a periciliary fluid of appropriate volume and composition to ensure proper mucociliary clearance (Randell and Boucher 2006; Boucher 2007). Adversely affected mucus leads to impaired mucociliary clearance from the submucosal glands and airway surface. The uncleared mucus then becomes a sink for bacterial binding, infection, and inflammation, thereby perpetuating a vicious cycle leading to further mucus secretion (Quinton 1999). This sequence of events is not restricted to the 40,000 individuals suffering from CF, but also occurs in more than 10 million patients suffering from COPD (chronic obstructive pulmonary disease) (Celli et al. 1995; O’Byrneh et al. 1999). Thus, impaired fluid secretion by the submucosal glands or surface epithelium hinders clearance from the glands and airway surface. Until recently, Cl− was considered to be the secreted anion responsible for fluid secretion in the airways. However, recent studies suggest that HCO3− secretion importantly contributes to the airway surface and submucosal gland microenvironments.

AIRWAY CELLS SECRETE BICARBONATE

Several early studies indicated that the short-circuit current (ISC), a measure of net electrolyte transport, across airway epithelia was not fully accounted for by the net movements of Na+ and Cl−. Instead, 35%–45% of the ISC had to be attributed to an additional ion species. Ion substitution studies revealed that HCO3− but not Cl− was required to observe a cAMP-stimulated increase in ISC in airway monolayers (Al-Bazzaz et al. 1979, 1981; Welsh 1983). These early observations prompted Smith and Welsh (1992) to investigate HCO3− secretion in normal and CF-cultured airway epithelia. The results of this investigation revealed that cAMP-stimulated HCO3− secretion across normal but not CF airway epithelia. These investigators went on to conclude that HCO3− exit at the apical membrane is through the Cl− channel, which is defectively regulated in CF epithelia. In addition, they suggested the possibility that a defect in HCO3− secretion may contribute to the pathophysiology of CF pulmonary disease.

The intracellular microelectrode studies of Willumsen and Boucher (Willumsen et al. 1992), although designed for a different intent, provided important insight regarding the driving forces acting on HCO3− and the question of why normal, but not, CF epithelia can secrete HCO3−. These investigators observed that the intracellular pHs of normal and CF airway cells were equal (normal = 7.15 ± 0.02 vs. CF = 7.11 ± 0.05), but that the apical membrane potentials (Vap) were different and of opposite polarity (normal = −19 ± 2 mV vs. CF = 3 ± 5 mV). This difference has been confirmed in several additional studies by Boucher and co-workers (Boucher et al. 1988; Willumsen et al. 1989a,b). Thus, at an extracellular HCO3− concentration of 25 mM, an extracellular and intracellular pCO2 of 40 mm Hg, and a pH of 7.15, there is an outwardly directed driving force for HCO3− secretion of 6 mV across the apical membrane of normal cells. In contrast, in CF cells, there is an inwardly directed driving force for HCO3− absorption of 16 mV across the apical membrane. Therefore, provided there is a conductive pathway to mediate the movement of HCO3− across the apical membrane, normal cells will secrete HCO3− and CF cells will absorb HCO3−. Although it is often suggested that impaired Cl− secretion must be corrected in CF, it is noteworthy, given the above Vap, that the usual extracellular and intracellular Cl−
concentrations, that the net driving force acting on Cl\(^-\) is in the absorptive direction for both normal (9 mV) and CF (31 mV) cells. Indeed, only after Na\(^+\) transport is down-regulated or inhibited with amiloride is it possible to observe Cl\(^-\) secretion in normal cells. It is generally accepted that the difference in \(V_{\text{ap}}\) between normal and CF cells is due to the loss of CFTR channels and the higher Na\(^+\) permeability in CF cells (Boucher et al. 1986, 1988; Boucher 1994a,b). Besides Cl\(^-\), CFTR also conducts HCO\(_3\)^\(-\), as shown in the studies of Gray et al. (1990) and Linsdell and coworkers (Linsdell et al. 1997; Tang et al. 2009; Li et al. 2011) and more recently by Ishiguro et al. (2009). Therefore, normal airway cells can secrete HCO\(_3\)\(^-\) through CFTR, whereas CF cells show a HCO\(_3\)\(^-\) impermeability. This reasoning leads us to assert that, in addition to abnormal Cl\(^-\) and Na\(^+\) transport, HCO\(_3\)\(^-\) transport is also dysfunctional in CF airway epithelia and that HCO\(_3\)\(^-\) secretion and not Cl\(^-\) secretion is critical for normal surface airway epithelial function.

## STUDIES WITH Calu-3 CELLS

A second line of evidence that HCO\(_3\)\(^-\) secretion in the airways may be more important than previously appreciated comes from the studies of Wine and coworkers (Lee et al. 1998) and the results on Calu-3 cells from my own group (Devor et al. 1999). Calu-3 cells are a human airway serous cell line developed by Wine and Widdicombe and coworkers (Shen et al. 1994). The Calu-3 cells were selected from among 12 lung adenocarcinomas as a cell line consistent with cell biological and electrophysiological characteristics of airway serous cells. Calu-3 cells form confluent monolayers with transepithelial resistances of several hundred ohm-centimeters squared (\(\Omega\) cm\(^2\)), express high levels of CFTR, and respond to both cAMP- and Ca\(^2+\)-mediated agonists with changes in net transepithelial ion transport as measured by \(I_{\text{SC}}\) (Finkbeiner et al. 1993; Shen et al. 1994). In addition, the Calu-3 cells produce several serous cell-associated proteins including lysozyme, lactoferrin, serine leukoprotease inhibitor (SLP1), secretory component, and mucins (MUC1 and MUC2) (Finkbeiner et al. 1993). The Calu-3 cells are now used by many laboratories as a serous cell model (Kelley et al. 1995; Grygorczyk et al. 1997; Liedtke et al. 1998; Al-Nakkash et al. 1999; Berger et al. 1999; Duszky et al. 1999; Illek et al. 1999; Ito et al. 1999; Waters et al. 1999). Our studies on Calu-3 cells showed that they secrete HCO\(_3\)\(^-\) rather than Cl\(^-\) in response to cAMP-mediated agonists. In effect, the Calu-3 cells function as one would expect for a pancreatic ductal cell; however, a cell line of the latter is thus far unavailable. Calu-3 cells express high levels of CFTR, as do serous cells of the submucosal glands of the native epithelium (Puchelle et al. 1992; Engelhardt et al. 1994) and pancreatic ductal cells (Marino et al. 1991). In addition, Calu-3 cells express Na\(^+:\)HCO\(_3\)\(^-\) cotransporter isoforms pNBC1 (SLC4A4, NBCe1) and NBC4 (SLC4A5, NBCe2) in the basolateral membrane (Kreindler et al. 2006). Studies from Case and coworkers on pancreatic ducts (Ishiguro et al. 1996a,b) and the studies of my laboratory on Calu-3 cells (Devor et al. 1999) suggest that HCO\(_3\)\(^-\) entry across the basolateral membrane is mediated by an NBC. If we assume that the transport phenotype expressed by Calu-3 cells accurately reflects native serous cells, then HCO\(_3\)\(^-\) secretion must be important in the physiology of submucosal glands. In agreement with this hypothesis, the studies of Ballard and coworkers have shown that inhibitors of HCO\(_3\)\(^-\) secretion, acetazolamide, a carbonic anhydrase inhibitor, and DIDS, an inhibitor of Cl\(^-\)\(\rightarrow\)HCO\(_3\)\(^-\) exchangers as well as NBCs, caused mucus obstruction of the submucosal glands in secretagogue-stimulated porcine distal bronchi (Inglis et al. 1997, 1998; Trout et al. 1998). Furthermore, bumetanide, an inhibitor of the Na\(^+\)-\(\rightarrow\)K\(^+\):2 Cl\(^-\) cotransporter (NKCC1) and thereby Cl\(^-\) secretion failed to inhibit cAMP-induced gland fluid secretion (Corrales et al. 1984). Consistent with these results, the forskolin-stimulated increase in \(I_{\text{SC}}\) in Calu-3 cells is insensitive to bumetanide but is inhibited by acetazolamide and DNDS, another inhibitor of NBCs (Devor et al. 1999). Collectively, these studies lead us to conclude that HCO\(_3\)\(^-\) secretion is important in the physiology of submucosal glands and the airway surface epithelium.
MODEL FOR ANION SECRETION IN AIRWAY CELLS

Our model for anion secretion in Calu-3 cells is illustrated in Figure 1. Forskolin-stimulated Calu-3 cells secrete HCO$_3^-$ by an electrogenic mechanism, that is, Cl$^-$-independent, serosal Na$^+$-dependent, serosal bumetanide-insensitive and inhibited by serosal disulfonic stilbene (DNDS) as judged by transepithelial currents, isotope fluxes, and the results of ion substitution, pharmacology, and pH studies (Devor et al. 1999). However, Calu-3 cells are not limited to the secretion of HCO$_3^-$. Instead, when stimulated by 1-EBIO (1-ethyl-2-benzimidazolinone), an activator of the basolateral membrane, Ca$^{2+}$-activated CTX-sensitive, K$^+$ channels (hIK1, KCNN4), Calu-3 cells secrete Cl$^-$ by an electrogenic bumetanide-sensitive mechanism, and HCO$_3^-$ secretion is diminished. Moreover, when stimulated by both forskolin and 1-EBIO, the secretion of HCO$_3^-$ is diminished and Cl$^-$ secretion dominates. To account for these results, we proposed the above model of anion secretion whereby CFTR serves as the cAMP/PKA-activated anion channel for both Cl$^-$ and HCO$_3^-$ exit across the apical membrane. Activation of CFTR alone tends to bring $V_{ap}$ to the equilibrium potential for Cl$^-$ ($E_{Cl}$), a value greater than the equilibrium potential for HCO$_3^-$ ($E_{HCO3}$), and thereby provides the driving force for HCO$_3^-$ exit across the apical membrane. Stimulation by cAMP (forskolin) alone leaves the basolateral membrane potential ($V_{bl}$) less hyperpolarized than the reversal potential of the DNDS-sensitive NBC ($E_{revNBC}$), and HCO$_3^-$ is secreted. Subsequent activation of hIk1 by 1-EBIO or cholinergic agonists hyperpolarizes $V_{bl}$ so that $V_{bl} > E_{revNBC}$ which inhibits HCO$_3^-$ uptake by an NBC but provides a driving force for Cl$^-$ secretion as $V_{ap}$ becomes $> E_{Cl}$.

The initial results that led to the discovery that Calu-3 cells secrete HCO$_3^-$ in response to cAMP stimulation and Cl$^-$ when hIK1 channels are activated by 1-EBIO are illustrated in Figure 2 (Devor et al. 1999). Calu-3 cells were grown on collagen-coated filters and studied by standard short-circuit current ($I_{SC}$) methods. In standard bath solutions of NaCl and NaHCO$_3$, the Calu-3 cells display a basal $I_{SC}$ of 13 ± 0.8 µA cm$^{-2}$ and a transepithelial resistance ($R_t$) of 353 ± 14 Ω cm$^2$ ($n = 216$ filters). Stimulation

Figure 1. Model of anion secretion in Calu-3 cells.

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with forskolin (2 μM) induced a damped oscillatory response that became stable and sustained after 5–10 min at a plateau value of 66 ± 4 μA cm⁻² and at a reduced Rₑ of 189 ± 7 Ω cm⁻² (n = 109). The subsequent addition of 1-EBIO (1 mM) caused a further increase in Iₛₑ to 114 ± 5 μA cm⁻² without any further significant decrease in Rₑ (173 ± 9 Ω cm⁻², n = 36). Unidirectional ion fluxes revealed that forskolin caused a fivefold increase in both the mucosal-to-serosal and serosal-to-mucosal fluxes of Cl⁻, but no net secretion of Cl⁻ (Fig. 2B). However, when the forskolin-stimulated monolayers were further stimulated with 1-EBIO, there was a further increase in the serosal-to-mucosal flux of Cl⁻, and the net secretion of Cl⁻ was nearly equal to the Iₛₑ. Moreover, whereas the forskolin-stimulated Iₛₑ and Cl⁻ fluxes were insensitive to bumetanide, bumetanide completely inhibited the Iₛₑ and the net secretion of Cl⁻ in forskolin plus 1-EBIO–treated monolayers (results not shown). Additional ion substitution studies revealed that the forskolin-stimulated Iₛₑ was Cl⁻ independent, HCO₃⁻ dependent, and serosal Na⁺ dependent. Pharmacological studies revealed that forskolin-stimulated Iₛₑ was DNDS, acetazolamide sensitive. Collectively, these studies led us to conclude that Calu-3 cells secrete HCO₃⁻ in response to cAMP stimulation. This conclusion was strongly supported by the observation that forskolin stimulation caused an alkalinization of the mucosal bathing solution of Calu-3 cells studied under open circuit conditions. In agreement with our estimates, Wine and coworkers (Irokawa et al. 2004), using a novel chamber to collect the fluid secreted by Calu-3 cells, showed that forskolin stimulated the secretion of a solution of ~80 mM HCO₃⁻. Moreover, when forskolin plus 1-EBIO was used to stimulate the cells, the fluid was no longer alkaline, and the HCO₃⁻ concentration fell to 17 mM (Irokawa et al. 2004).

**CFTR Cl⁻-DEPENDENT SECRETION**

The studies with 1-EBIO reveal that Calu-3 cells are not limited to HCO₃⁻ secretion but can also secrete Cl⁻. We used 1-EBIO in these studies because it causes a sustained activation of the basolateral membrane Ca²⁺-activated, CTX-sensitive K⁺ channels (hIK1) (Devor et al. 1996a,b; Syme et al. 2000). Although relatively high concentrations of 1-EBIO are required (1 mM), subsequent studies with newly synthesized derivatives that we have prepared with 100-fold higher affinities produce the same effects (Singh et al. 1999). Wine and coworkers (Lee et al. 1998) also observed similar results.
using thapsigargin to elevate intracellular Ca\(^{2+}\) and activate hIK channels. These channels would normally be activated by Ca\(^{2+}\)-mediated agonists such as acetylcholine or substance-P. However, the former does not cause sustained increases in intracellular Ca\(^{2+}\) and \(I_{SC}\). In addition to causing Cl\(^{-}\) secretion, the activation of hIK1 channels diminishes HCO\(_3\)\(^{-}\) secretion. These results combined with the observations that forskolin-stimulated HCO\(_3\)\(^{-}\) secretion required serosal Na\(^{+}\) and was inhibited by DNDS led us to propose that the influx of HCO\(_3\)\(^{-}\) across the basolateral membrane was mediated by an electrogenic NBC that carries two or more HCO\(_3\)\(^{-}\) ions for each Na\(^{+}\) ion. Activation of basolateral membrane K\(^{+}\) channels tends to hyperpolarize \(V_{bl}\) and inhibit NBC-dependent HCO\(_3\)\(^{-}\) influx. The hyperpolarization of \(V_{bl}\) also activates the basolateral membrane NaK2Cl-co-transporter (Haas and Forbush 2000), allowing for the influx of Cl\(^{-}\) and its net secretion.

Therefore, the activation and inactivation of basolateral membrane K\(^{+}\) channels provides a means of switching between HCO\(_3\)\(^{-}\) and Cl\(^{-}\) secretion across airway epithelia as illustrated in Figure 1.

**MICROELECTRODE AND IMPEDANCE ANALYSIS STUDIES**

To obtain a better understanding of the conductances and driving forces involved in these different modes of anion secretion in Calu-3 cells, we performed microelectrode and impedance analysis experiments (Tamada et al. 2001). The results of these experiments are summarized in cell models shown in Figure 3. We were able to maintain microelectrode impalements for 10–30 min on a routine basis in Calu-3 cells, which allowed us to monitor the same cell under control, forskolin, and forskolin plus 1-EBIO–stimulated conditions. Cells were

![Cell models of unstimulated control cells, forskolin-stimulated cells secreting HCO\(_3\)\(^{-}\), and forskolin plus 1-EBIO cells secreting Cl\(^{-}\). See text for details.](http://perspectivesinmedicine.cshlp.org/)

**Figure 3.**
studied under open circuit conditions, and the transepithelial voltage \( (V_t) \) and resistance \( (R_t) \) as well as the \( V_{ap} \) were measured. The apical fractional resistance \( (F_{Rap}) \) was calculated from the \( \Delta V_t \) and the \( \Delta V_{ap}/\Delta V_t \) ratio in response to a 50-\( \mu \)A transepithelial pulse and the \( V_{bl} \) from the \( V_t \) and \( V_{ap} \) values. Forskolin caused a hyperpolarization of \( V_t \) and decreased \( R_t \) similar to the changes seen in the short-circuit current experiments yielding equivalent current changes \( (I_{eq}) \) similar to the previously reported \( I_{SC} \) values of \( \sim 65 \mu \text{A/cm}^2 \). Forskolin depolarized both \( V_{ap} \) and \( V_{bl} \). \( V_{ap} \) decreased from a control value in unstimulated cells of \( -54.3 \text{ mV} \) to \( -21.3 \text{ mV} \) and \( V_{bl} \) from \( -60.5 \text{ mV} \) to \( -41.2 \text{ mV} \). Consistent with the activation of an apical membrane conductance, the \( F_{Rap} \) decreased from 0.55 to 0.074 with forskolin stimulation. Double barrel voltage and pH electrodes were used to obtain estimates of the intracellular pH \( (\text{pH}_i) \). Forskolin increased \( \text{pH}_i \) from a control value of 7.02 to 7.16. Assuming a \( pCO_2 \) of 40 mm Hg, the intracellular \( \text{HCO}_3^- \) concentration increased from 11 mM in control cells to 13.5 mM in forskolin-stimulated cells. Thus, forskolin stimulated a driving force of 5.8 mV for \( \text{HCO}_3^- \) exit from the cell. Consistent with the dramatic decrease in the \( F_{Rap} \) impedance analysis showed that the apical membrane resistance fell to 14 \( \text{mS/cm}^2 \) in forskolin-stimulated cells corresponding to an apical membrane conductance of 71 \( \text{mS/cm}^2 \).

With a driving force of 5.8 mV, only 12 \( \text{mS/cm}^2 \) of the apical membrane conductance of 71 \( \text{mS/cm}^2 \) is needed to account for the observed \( \text{HCO}_3^- \) current of 65 \( \mu \text{A/cm}^2 \). The conductance ratio of 12 \( \text{mS/cm}^2 \) to 71 \( \text{mS/cm}^2 \) is 0.17, and this too is consistent with the reported \( \text{HCO}_3^- \) conductance compared with \( \text{Cl}^- \) of the apical membrane of Calu-3 cells \( (\text{Illek et al. 1999}) \) as well as \( \text{CFTR} \) \( (\text{Linsdell et al. 1997; Tang et al. 2009; Man-song et al. 2010}) \). It must also be noted that a driving force of 5.8 mV does not explain the reported 80 mm concentration of \( \text{HCO}_3^- \) in the thin film experiments \( (\text{Devor et al. 1999; Irokawa et al. 2004}) \).

It is noteworthy that a driving force of 32.4 mV exists for \( \text{HCO}_3^- \) exit in unstimulated control cells, and yet not until \( \text{CFTR} \) is activated is \( \text{HCO}_3^- \) secreted, and this secretion is driven by a much lower driving force (5.8 mV). Thus, if alternative \( \text{HCO}_3^- \) transporters are present in the apical membrane, they are not active until the cells are stimulated with forskolin. In addition, the intracellular \( \text{HCO}_3^- \) concentration is actually increased in forskolin-stimulated cells, indicating that the secreted \( \text{HCO}_3^- \) is replenished. We surmise that \( \text{HCO}_3^- \) entry via a basolateral membrane \( \text{NBC} \) is activated by depolarization of the basolateral membrane. Given a reversal potential for an \( \text{NBC} \) with a stoichiometry of 1 \( \text{Na}^+ / 2 \text{HCO}_3^- \) of nearly \( -90 \text{ mV} \), we suggested that the \( \text{NBC} \) that mediates \( \text{HCO}_3^- \) entry has a stoichiometry of 1 \( \text{Na}^+ / 3 \text{HCO}_3^- \) with a reversal potential of approximately \( -60 \text{ mV} \). Depolarization of \( V_{bl} \) from \( -61.5 \text{ mV} \) in unstimulated cells to \( -44 \text{ mV} \) would activate an \( \text{NBC} \) with a 1:3 stoichiometry. Addition of \( \text{DNDS} \) to the basolateral side caused a depolarization of 8.5 mV in forskolin-stimulated cells but not in unstimulated cells. Replacement of \( \text{HCO}_3^- \) with \( \text{HEPES} \) also depolarized \( V_{bl} \) by 8.5 mV \( (\text{T Tamada and RJ Bridges, unpubl.}) \). These results are consistent with the activation of an electrogenic, \( \text{DNDS} \)-sensitive basolateral membrane \( \text{NBC} \) in forskolin-stimulated cells. Confirmation of the exact stoichiometry of the \( \text{NBC} \) will require measurements of the intracellular \( \text{Na}^+ \) activity.

The microelectrode and impedance studies revealed that 1-\( \text{EBIO} \) hyperpolarized \( V_{bl} \) and \( V_{ap} \) when added to forskolin-stimulated cells. \( V_{bl} \) hyperpolarized from \( -41.2 \text{ mV} \) to \( -61.1 \text{ mV} \) and \( V_{ap} \) from \( -21.3 \text{ mV} \) to \( -27.4 \text{ mV} \) when 1-\( \text{EBIO} \) was added to the forskolin-stimulated cells. The hyperpolarization caused by 1-\( \text{EBIO} \) was reversed by the addition of \( \text{hIK1} \) blockers \( (\text{T Tamada and RJ Bridges, unpubl.}) \). Impedance analysis confirmed that 1-\( \text{EBIO} \) activated a basolateral membrane conductance, and the reversal of this conductance increased with \( \text{hIK1} \) blockers. 1-\( \text{EBIO} \) also decreased \( \text{pH}_i \) from 7.16 to 7.01, and intracellular \( \text{HCO}_3^- \) from 13.5 to 9.8 mM. \( V_{bl} \) was no longer sensitive to \( \text{DNDS} \) or to serosal \( \text{HCO}_3^- \) replacement with \( \text{HEPES} \). However, bumetanide decreased \( V_{bl} \), and as noted above, the current was almost fully accounted for by \( \text{Cl}^- \) secretion in forskolin plus 1-\( \text{EBIO} \)-stimulated cells. These results are
consistent with the activation of basolateral membrane hK-1 potassium channels. For an NBC with a 1:3 Na⁺-to-HCO₃⁻ stoichiometry, the hyperpolarization of \( V_{bl} \) to \(-61.1 \) exceeds the reversal potential of the NBC and thereby would allow HCO₃⁻ exit from the cell on the NBC. Consequently, both \( p_H \) and intracellular \( HCO_3^- \) decrease. Even though the \( p_H \) and intracellular \( HCO_3^- \) have decreased, a driving force of 3.0 mV still exists for \( HCO_3^- \) exit across the apical membrane. If cell metabolism produces sufficient \( HCO_3^- \), then \( HCO_3^- \) secretion should persist. Indeed, acetazolamide causes a small decrease in the \( I_{SC} \) in forskolin plus 1-EBIO-stimulated cells.

1-EBIO also appears to activate the basolateral membrane NKCC cotransporter. Activation of the NKCC may result from the hyperpolarization of \( V_{bl} \) as well as cell shrinkage due to the loss of intracellular \( Cl^- \) and K⁺ (Haas and Forbush 2000). Impedance analysis and cell height measurements confirm that Calu-3 cells shrink when 1-EBIO is added to forskolin-stimulated cells (RJ Bridges and W van Driessche, unpubl.). Addition of 1-EBIO also hyperpolarizes \( V_{ap} \) in forskolin-stimulated cells. If intracellular \( Cl^- \) were to remain unchanged in forskolin- and forskolin plus 1-EBIO-stimulated cells, a driving force of 6.1 mV would exist for \( Cl^- \) exit across the apical membrane. A 6.1-mV driving force across an apical membrane conductance of 71 mS/cm² should result in a current of 433 \( \mu A/cm^2 \). Because we observe a \( Cl^- \) current of only \( \sim 140 \) \( \mu A/cm^2 \), we predict that the intracellular \( Cl^- \) concentration must decrease by 8 mM when 1-EBIO is added to the forskolin-stimulated cells. Unfortunately, we were unsuccessful in our attempts to fabricate a double barrel voltage and chloride-sensitive microelectrode to test this hypothesis.

**SUMMARY**

Calu-3 cells, an airway serous cell model with high levels of CFTR expression, secrete \( HCO_3^- \) in response to a cAMP-mediated agonist but can be stimulated to secrete \( Cl^- \) with a basolateral membrane K⁺ channel-activating agonist such as 1-EBIO. Activation of CFTR by forskolin results in a very high apical membrane conductance and a depolarization of \( V_{ap} \) to a value that approaches \( E_{Cl^-} \). With \( V_{ap} \) at \( E_{Cl^-} \), there is a net driving force for \( HCO_3^- \) exit from the cell across the apical membrane. Because of this driving force and the very high apical membrane conductance, \( HCO_3^- \) can be secreted from the cells despite the lower conductance of CFTR for \( Cl^- \) compared with \( Cl^- \). The very high apical membrane anion conductance also results in a depolarization of \( V_{bl} \) and the activation of a basolateral membrane NBC. Secreted \( HCO_3^- \) is supplied by the basolateral entry of \( HCO_3^- \) by this NBC. Thus, the activation of CFTR sets the \( V_{ap} \) at \( E_{Cl^-} \), provides the driving force for \( HCO_3^- \) exit, and results in the activation of a basolateral membrane NBC. Activation of basolateral membrane K⁺ channels hyperpolarizes \( V_{bl} \) and \( V_{ap} \). The hyperpolarization of \( V_{ap} \) provides a driving force for \( Cl^- \) secretion. The hyperpolarization of \( V_{ap} \) results in an inhibition of the NBC and the activation of the NKCC. Therefore, the switch from \( HCO_3^- \) secretion to \( Cl^- \) secretion is mediated by the activity of the basolateral K⁺ channel and which of the basolateral membrane cotransporters is active, NBC or the NKCC. Depending on which of these cotransporters is active, CFTR will secret either \( HCO_3^- \) or \( Cl^- \).

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