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 hK-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 hK-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_2O 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,

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and enzymes that must be flushed from the glands onto the airway surface epithelium (Basbaum 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'Byrne 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 HCO_3^- secretion importantly contributes to the airway surface and submucosal gland microenvironments.

AIRWAY CELLS SECRETE BICARBONATE

Several early studies indicated that the short-circuit current (I_{SC}), 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 I_{SC} had to be attributed to an additional ion species. Ion substitution studies revealed that HCO_3^- but not Cl^- was required to observe a cAMP-stimulated increase in I_{SC} in airway monolayers (Al-Bazzaz et al. 1979, 1981; Welsh 1983). These early observations prompted Smith and Welsh (1992) to investigate HCO_3^- secretion in normal and CF-cultured airway epithelia. The results of this investigation revealed that cAMP-stimulated HCO_3^- secretion across normal but not CF airway epithelia. These investigators went on to conclude that HCO_3^- 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 HCO_3^- 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 HCO_3^- and the question of why normal, but not, CF epithelia can secrete HCO_3^- . 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 (V_{ap}) 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 HCO_3^- concentration of 25 mM, an extracellular and intracellular pCO_2 of 40 mm Hg, and a pH of 7.15, there is an outwardly directed driving force for HCO_3^- secretion of 6 mV across the apical membrane of normal cells. In contrast, in CF cells, there is an inwardly directed driving force for HCO_3^- absorption of 16 mV across the apical membrane. Therefore, provided there is a conductive pathway to mediate the movement of HCO_3^- across the apical membrane, normal cells will secrete HCO_3^- and CF cells will absorb HCO_3^- . Although it is often suggested that impaired Cl^- secretion must be corrected in CF, it is noteworthy, given the above V_{ap} s and 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_{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 ($\Omega \text{ cm}^2$), 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_{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; Duszyk 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 $\text{Na}:\text{HCO}_3^-$ 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 $\text{Cl}^-:\text{HCO}_3^-$ 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 $\text{Na}^+:\text{K}^+:2 \text{Cl}^-$ 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_{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.

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**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_{HCO_3}), 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 $V_{\text{bl}} > E_{\text{revNBC}}$, which inhibits HCO_3^- uptake by an NBC but provides a driving force for Cl^- secretion as V_{ap} becomes $> E_{\text{Cl}}$.

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 $13 \pm 0.8 \mu\text{A cm}^{-2}$ and a transepithelial resistance (R_t) of $353 \pm 14 \Omega \text{ cm}^2$ ($n = 216$ filters). Stimulation

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

Figure 2. Effect of forskolin (2 μM) and 1-EBIO (1 mM) on I_{sc} and Cl^- fluxes across Calu-3 cells. Ion fluxes are given as absolute values. Forskolin caused a fivefold increase in each of the unidirectional fluxes without any net secretion of Cl^- . Control data are not shown. (Figure is from Devor et al. 1999; reprinted, with permission, from the author.)

with forskolin (2 μM) induced a damped oscillatory response that became stable and sustained after 5–10 min at a plateau value of $66 \pm 4 \mu\text{A cm}^{-2}$ and at a reduced R_t of $189 \pm 7 \Omega \text{ cm}^{-2}$ ($n = 109$). The subsequent addition of 1-EBIO (1 mM) caused a further increase in I_{sc} to $114 \pm 5 \mu\text{A cm}^{-2}$ without any further significant decrease in R_t ($173 \pm 9 \Omega \text{ cm}^{-2}$, $n = 36$). 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 I_{sc} . Moreover, whereas the forskolin-stimulated I_{sc} and Cl^- fluxes were insensitive to bumetanide, bumetanide completely inhibited the I_{sc} 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 I_{sc} was Cl^- independent, HCO_3^- dependent, and serosal Na^+ dependent. Pharmacological studies revealed that forskolin-stimulated I_{sc} was DNDS and acetazolamide sensitive. Collectively, these studies led us to conclude that Calu-3 cells secrete HCO_3^- in response to cAMP stim-

ulation. 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 $\sim 80 \text{ mM HCO}_3^-$. Moreover, when forskolin plus 1-EBIO was used to stimulate the cells, the fluid was no longer alkaline, and the HCO_3^- 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 HCO_3^- secretion but can also secrete Cl^- . We used 1-EBIO in these studies because it causes a sustained activation of the basolateral membrane Ca^{2+} -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

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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

Figure 3. Cell models of unstimulated control cells, forskolin-stimulated cells secreting HCO_3^- , and forskolin plus 1-EBIO cells secreting Cl^- . See text for details.

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 ΔV_t and the $\Delta V_{ap}/\Delta V_t$ ratio in response to a 50- μ 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 $\sim 65 \mu\text{A}/\text{cm}^2$. Forskolin depolarized both V_{ap} and V_{bl} . V_{ap} decreased from a control value in unstimulated cells of -54.3 mV to -21.3 mV and V_{bl} from -60.5 mV to -41.2 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 (pH_i). Forskolin increased pH_i from a control value of 7.02 to 7.16. Assuming a pCO_2 of 40 mm Hg, the intracellular 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 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 $14 \Omega \text{ cm}^2$ in forskolin-stimulated cells corresponding to an apical membrane conductance of $71 \text{ mS}/\text{cm}^2$. With a driving force of 5.8 mV, only $12 \text{ mS}/\text{cm}^2$ of the apical membrane conductance of $71 \text{ mS}/\text{cm}^2$ is needed to account for the observed HCO_3^- current of $65 \mu\text{A}/\text{cm}^2$. The conductance ratio of $12 \text{ mS}/\text{cm}^2$ to $71 \text{ mS}/\text{cm}^2$ is 0.17, and this too is consistent with the reported HCO_3^- conductance compared with Cl^- of the apical membrane of Calu-3 cells (Illek et al. 1999) as well as CFTR (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 HCO_3^- in the thin film experiments (Devor et al. 1999; Irokawa et al. 2004).

It is noteworthy that a driving force of 32.4 mV exists for HCO_3^- exit in unstimulated control cells, and yet not until CFTR is activated is HCO_3^- secreted, and this secretion is driven

by a much lower driving force (5.8 mV). Thus, if alternative HCO_3^- transporters are present in the apical membrane, they are not active until the cells are stimulated with forskolin. In addition, the intracellular HCO_3^- concentration is actually increased in forskolin-stimulated cells, indicating that the secreted HCO_3^- is replenished. We surmise that HCO_3^- entry via a basolateral membrane NBC is activated by depolarization of the basolateral membrane. Given a reversal potential for an NBC with a stoichiometry of 1 Na^+ to 2 HCO_3^- of nearly -90 mV , we suggested that the NBC that mediates HCO_3^- entry has a stoichiometry of 1 Na^+ to 3 HCO_3^- with a reversal potential of approximately -60 mV . Depolarization of V_{bl} from -61.5 mV in unstimulated cells to -44 mV would activate an NBC with a 1:3 stoichiometry. Addition of DNDS to the basolateral side caused a depolarization of 8.5 mV in forskolin-stimulated cells but not in unstimulated cells. Replacement of HCO_3^- with HEPES also depolarized V_{bl} by 8.5 mV (T Tamada and RJ Bridges, unpubl.). These results are consistent with the activation of an electrogenic, DNDS-sensitive basolateral membrane NBC in forskolin-stimulated cells. Confirmation of the exact stoichiometry of the NBC will require measurements of the intracellular Na^+ activity.

The microelectrode and impedance studies revealed that 1-EBIO hyperpolarized V_{bl} and V_{ap} when added to forskolin-stimulated cells. V_{bl} hyperpolarized from -41.2 mV to -61.1 mV and V_{ap} from -21.3 mV to -27.4 mV when 1-EBIO was added to the forskolin-stimulated cells. The hyperpolarization caused by 1-EBIO was reversed by the addition of hIK1 blockers (T Tamada and RJ Bridges, unpubl.). Impedance analysis confirmed that 1-EBIO activated a basolateral membrane conductance, and the reversal of this conductance increased with hIK1 blockers. 1-EBIO also decreased pH_i from 7.16 to 7.01, and intracellular HCO_3^- from 13.5 to 9.8 mM. V_{bl} was no longer sensitive to DNDS or to serosal HCO_3^- replacement with HEPES. However, bumetanide decreased V_t , and as noted above, the current was almost fully accounted for by Cl^- secretion in forskolin plus 1-EBIO-stimulated cells. These results are

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consistent with the activation of basolateral membrane hK-1 potassium channels. For an NBC with a 1:3 Na^+ -to- HCO_3^- stoichiometry, the hyperpolarization of V_{bl} to -61.1 exceeds the reversal potential of the NBC and thereby would allow HCO_3^- exit from the cell on the NBC. Consequently, both pH_i and intracellular HCO_3^- decrease. Even though the pH_i 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 $\mu\text{A}/\text{cm}^2$. Because we observe a Cl^- current of only ~ 140 $\mu\text{A}/\text{cm}^2$, 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 for-

skolin 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 HCO_3^- 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_{bl} 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 secrete either HCO_3^- or Cl^- .

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