Microelectrode and Impedance Analysis of Anion Secretion in Calu-3 Cells

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Summary

Calu-3 cells secrete HCO₃⁻ in response to cAMP agonists but can be stimulated to secrete Cl⁻ with K⁺ channel activating agonists. Microelectrode and impedance analysis experiments were performed to obtain a better understanding of the conductances and driving forces involved in these different modes of anion secretion in Calu-3 cells. Microelectrode studies revealed apical and basolateral membrane depolarizations upon the addition of forskolin (Vₑₐₚ −52 mV vs. −21 mV; Vₑₜ −60 mV vs. −44 mV) that paralleled the hyperpolarization of the mucosal negative transepithelial voltage (Vₜ −8 mV vs. −23 mV). These changes were accompanied by a decrease in the apical membrane fractional resistance (Fₑₐₚ) from approximately 0.50 to 0.08, consistent with the activation of an apical membrane conductance. The subsequent addition of 1-ethyl-2-benzimidazolinone (1-EBIO), a K⁺ channel activator, hyperpolarized Vₑₐₚ to −27 mV, Vₑₚ to −60 mV and Vₑₜ to −33 mV. Impedance analysis revealed the apical membrane resistance (Rₑₐₚ) of the forskolin-stimulated cells was less than 20 Ω cm², indeed in most monolayers Rₑₐₚ fell to less than 5 Ω cm². The impedance derived estimate of the basolateral membrane resistance (Rₑₚ) was approximately 170 Ω cm² in forskolin treated cells and fell to 50 Ω cm² with the addition of 1-EBIO. Using these values for the Rₑₚ and the Fₑₐₚ value of 0.08 yields a Rₑₐₚ of approximately 14 Ω cm² in the presence of forskolin and 4 Ω cm² in the presence of forskolin plus 1-EBIO. Thus, by two independent methods, forskolin-stimulated Calu-3 cells are seen to have a very high apical membrane conductance of 50 to 200 mS/cm². Therefore, we would assert that even at one-tenth the anion selectivity for Cl⁻, this high conductance could support the conductive exit of HCO₃⁻ across the apical membrane. We further propose that this high apical membrane conductance serves to clamp the apical membrane potential near the equilibrium potential for Cl⁻ and thereby provides the driving force for HCO₃⁻ secretion in forskolin-stimulated Calu-3 cells. The hyperpolarization of Vₑₚ and Vₑₚ caused by 1-EBIO provides a driving force for Cl⁻ exit across the apical membrane, inhibits the influx of HCO₃⁻ on the Na⁺:HCO₃⁻ cotransporter across the basolateral membrane, activates the basolateral membrane Na⁺:K:2Cl⁻ cotransporter and thereby provides the switch from HCO₃⁻ secretion to Cl⁻ secretion.

Our model for anion secretion in Calu-3 cells is illustrated in Figure 1. We have demonstrated forskolin stimulated Calu-3 cells secrete HCO₃⁻ by an electrogenic, Cl⁻ independent, serosal Na⁺-dependent, serosal bumetanide-insensitive
and serosal disulfonic stilbene (DNDS)-sensitive mechanism as judged by transepithelial currents, isotope fluxes, and the results of ion substitution, pharmacology and pH studies [1]. However, Calu-3 cells are not limited to the secretion of HCO$_3^-$

Instead, when stimulated by 1-EBIO, an activator of basolateral membrane, Ca$^{2+}$-activated, charybdotoxin-sensitive K$^+$ channels (K$_{Ca}$), the 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. A similar switch from HCO$_3^-$ secretion to Cl$^-$ secretion in Calu-3 cells was reported by Lee et al. [2] using thapsigargin to activate K$_{Ca}$ channels. To account for these results, we proposed a model of anion secretion whereby cystic fibrosis transmembrane conductance regulator (CFTR) serves as the cAMP/protein kinase A (cAMP/PKA) activated anion channel for both Cl$^-$ and HCO$_3^-$ exit across the apical membrane. The driving force for HCO$_3^-$ or Cl$^-$ exit across the apical membrane is equal to the apical membrane potential ($V_{ap}$) minus the equilibrium potential for HCO$_3^-$ or Cl$^-$, $E_{HCO_3}$ or $E_{Cl}$, respectively. Activation of CFTR alone will tend to bring $V_{ap}$ to $E_{Cl}$, a value that is predicted to be greater than $E_{HCO_3}$ and thus provides the driving force for HCO$_3^-$ exit. Stimulation by cAMP (forskolin) alone leaves the basolateral membrane potential ($V_{bl}$) less hyperpolarized than the reversal potential of the 4,4'-dinitrostilben-2,2'-disulfonic acid (DNDS)-sensitive NBC ($E_{RevNBC}$) and HCO$_3^-$ is secreted. Subsequent activation of K$_{Ca}$ by 1-EBIO or cholinergic agonists hyperpolarizes $V_{bl}$ so that $V_{bl}$ greater than $E_{RevNBC}$, and this inhibits HCO$_3^-$ uptake by the NBC but it provides the driving force for Cl secretion because $V_{ap}$ becomes greater than $E_{Cl}$. Whether cAMP/PKA activates the NBC directly is unknown. The Na$^+:$HCO$_3^-$ stoichiometry of the Calu-3 cell NBC is also unknown. At a Na$^+:$HCO$_3^-$ stoichiometry of 1:3, $E_{RevNBC}$ is –49 mV and at a stoichiometry of 1:2 $E_{RevNBC}$ is –84 mV. Thus, the model in Figure 1 leads to several readily testable predictions. To begin to test these hypotheses, we have performed microelectrode and impedance studies using the Calu-3 cells as an experimental model of airway serous cells.

Calu-3 cells were grown on Snapwell filters as previously described [1] and studied in a horizontal chamber that allowed for the impalement of the cells with a microelectrode from the apical side. The transepithelial potential ($V_T$) and $V_{ap}$ were recorded from cells maintained under open circuit conditions. A 50 µA transepithelial bipolar pulse was passed every 20 seconds and the transepithelial resistance ($R_T$) and the apical fractional resistance ($F_{Rap}$) were calculated from the $\Delta V_T$ and $\Delta V_{ap}/\Delta V_T$ ratio respectively as previously described [3]. Both the apical and basolateral surfaces were continuously perfused with a warm (37 °C) gassed (95%/5%, O$_2$/CO$_2$) solution. Results from a typical microelectrode experiment are shown in Figure 2. We were able to maintain the microelectrode impalement for 10 to 30 minutes on a routine basis. This allowed us to monitor the same cell under control, forskolin (2 µM) and forskolin plus 1-EBIO (1 mM) stimulated conditions. In the experiment shown in Figure 2 $V_T$ was approximately –5.5 mV, mucosal side negative, under control conditions and hyperpolarized to –22 mV with forskolin stimulation and further hyperpolarized to –35 mV with the subsequent
addition of forskolin plus 1-EBIO. The $R_T$ decreased from a control value of 530 $\Omega$ cm$^2$ to 320 $\Omega$ cm$^2$ with forskolin and to 235 $\Omega$ cm$^2$ with forskolin plus 1-EBIO. Using these $V_T$ and $R_T$ values one obtains an equivalent short circuit current of 10 $\mu$A/cm$^2$, 68 $\mu$A/cm$^2$ and 148 $\mu$A/cm$^2$ for the control, forskolin and forskolin plus 1-EBIO conditions, respectively. These results are in excellent agreement with the results obtained under short circuit current conditions where it was demonstrated that Calu-3 cells secrete HCO$_3^-$ when stimulated with forskolin and Cl$^-$ when stimulated with forskolin plus 1-EBIO [1].

Panel B of Figure 2 is the voltage measured by the microelectrode which upon impalement has a value of approximately $-53$ mV that improved to a value of $-59$ mV after a few minutes. Upon the addition of forskolin, $V_{ap}$ depolarized to a value of $-22$ mV and then repolarized to a value of $-28$ mV upon the subsequent addition of forskolin plus 1-EBIO. Panel C of Figure 2 is a plot of the $F_{Rap}$ from the same experiment and shows that $F_{Rap}$ fell from a control value of approximately 0.6 to approximately 0.1 upon stimulation with forskolin and was unchanged when forskolin plus 1-EBIO was added. Shortly after the addition of 1-EBIO the impalement was lost. This was frequently observed whenever 1-EBIO was added and is an effect we speculate may be due to cell shrinkage since advancing the electrode often reestablished the impalement.

Figure 3 summarizes the results of 25 similar experiments. As already noted the hyperpolarization of $V_T$ is consistent with the secretion of HCO$_3^-$ in forskolin stimulated cells and Cl$^-$ in forskolin plus 1-EBIO stimulated cells. These changes in $V_T$ reflect the activation of an apical membrane conductance so that $V_{ap}$ depolarized and $F_{Rap}$ decreased upon the addition of forskolin. $V_{bl}$ also depolarized from a control value of $-60\pm1.7$ mV to $-44\pm1.3$ mV in forskolin stimulated cells. The depolarization of $V_{bl}$ with forskolin can be explained by an
activation of an apical membrane anion conductance that dominates the total cellular conductance. Support for this notion is reflected in the remarkably low $F_{R_{ap}}$ observed in the forskolin stimulated cells and this will be further supported by the impedance results given below. Our model predicts $V_{ap}$ should be near $E_{Cl}$ in forskolin stimulated cells. Ideally, one should measure $V_{ap}$ and the intracellular $Cl^-$ activity using a double barreled microelectrode and these experiments are in progress. Based on the observation that $Cl^-$ is not secreted by forskolin stimulated cells, the measured $V_{ap}$ allows one to predict an intracellular $Cl^-$ activity of approximately 40 mM in the forskolin stimulated Calu-3 cells. This estimate of the intracellular $Cl^-$ activity agrees rather well with the measured values in other airway epithelial cells [4]. Perhaps more importantly the measured value of $-21.5\pm 1.3$ mV for $V_{ap}$ in forskolin stimulated cells is less than the $E_{RevNBC}$ of $-49$ mV and $-84$ mV for NBCs with $1:3$ or $1:2$ $Na^+:HCO_3^-$ stoichiometries and thus would allow $HCO_3^-$ to enter the cell on a basolateral membrane NBC. Therefore, the observed effects of forskolin on $V_T$, $V_{ap}$, $V_{bl}$ and $F_{R_{ap}}$ are consistent with the net secretion of $HCO_3^-$ by the mechanism proposed in our model.

When 1-EBIO is added to the forskolin stimulated cells, $HCO_3^-$ secretion is inhibited and $Cl^-$ secretion is stimulated [1]. The addition of 1-EBIO was observed to cause $V_{ap}$ and $V_{bl}$ to repolarize and these changes are the expected changes for the activation of basolateral membrane $K^+$ channels. The repolarization of $V_{ap}$ would increase the driving force for conductive anion exit across the apical. This is true for both $HCO_3^-$ and $Cl^-$ exit and therefore the change in $V_{ap}$ can not explain the switch from $HCO_3^-$ secretion to $Cl^-$ secretion. However, 1-EBIO was seen to cause $V_{bl}$ to repolarize from $-44\pm 1.3$ mV to $-60\pm 2.3$ mV. At a $V_{bl}$ of $-60$ mV an NBC with a $Na^+:HCO_3^-$ stoichiometry of $1:3$ ($E_{RevNBC} = -49$ mV) would be inhibited and $HCO_3^-$ would actually be expected to leave the cell rather then enter across the basolateral membrane. In contrast, if the $Na^+:HCO_3^-$ stoichiometry were $1:2$ ($E_{RevNBC} = -84$ mV) $HCO_3^-$ would still enter the cell and secretion of $HCO_3^-$ should continue. Thus, based on a measured $V_{bl}$ of $-60$ mV and the observation that $HCO_3^-$ secretion is inhibited when Calu-3 cells are stimulated by forskolin plus 1-EBIO, our results suggest the NBC responsible for $HCO_3^-$ entry has a $Na^+:HCO_3^-$ stoichiometry of $1:3$. This is a surprising outcome since the measured stoichiometries of the two NBC isovariants we have detected in Calu-3 cells, the kidney and pancreatic NBCs [5] both have $Na^+:HCO_3^-$ stoichiometries of $1:2$ when expressed in Xenopus oocytes [5, 6, 7]. Therefore, there must exist an alternative NBC

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**Figure 3.** Summary of the changes in transepithelial voltage ($V_T$), apical membrane potential ($V_{ap}$), basolateral membrane potential ($V_{bl}$) and apical membrane fractional resistance. Studies were performed as described in Figure 2. Values are the means ± SEM for n=25 monolayers.
with a 1:3 Na\(^{+}\):HCO\(_3\)\(^{-}\) stoichiometry that is expressed in the Calu-3 cells. Alternatively, the kidney or pancreatic NBC isovariants may be subjected to some form of regulation that alters the Na\(^{+}\):HCO\(_3\)\(^{-}\) stoichiometry from 1:2 to 1:3 when expressed in Calu-3 cells. Additional studies are required to clarify this point.

The remarkably low F\(_{\text{Rap}}\) value of 0.1 we observed in the forskolin stimulated Calu-3 cell monolayer prompted us to obtain an independent estimate of the apical and basolateral membrane resistances in the Calu-3 cells. To obtain these values we elected to perform impedance analysis studies. The impedance measurements were made on short-circuit Calu-3 cells grown as described above and given in detail elsewhere [1]. The transepithelial impedance was measured in response to a series of 100 sine waves over a frequency range of 1 Hz to 22 kHz as previously described [8, 9]. The impedance values are presented as Nyquist plots and were fit to the equations describing the equivalent electric circuit shown in Figure 4 to obtain estimates of the apical and basolateral membrane resistances (R\(_{\text{ap}}\), R\(_{\text{bl}}\)) and capacitances (C\(_{\text{ap}}\), C\(_{\text{bl}}\)). Only the resistance values will be considered here. The resistance of the paracellular pathway (R\(_{\text{P}}\)) was estimated from the y-intercept of a G\(_{\text{T}}\) (transepithelial conductance) versus I\(_{\text{SC}}\) plot [10] and was assumed to be constant under the different experimental conditions.

Shown in Figure 5A is an I\(_{\text{SC}}\) trace of a Calu-3 cell monolayer under control, forskolin (2 µM), 1-EBIO (1 mM) and charybotoxin (CTX) (50 nM) conditions. As previously reported [1], the Calu-3 cells display a control I\(_{\text{SC}}\) of approximately 8 µA/cm\(^2\). Addition of forskolin caused an increase in I\(_{\text{SC}}\) to 76 µA/cm\(^2\) and 1-EBIO further increased the I\(_{\text{SC}}\) to 130 µA/cm\(^2\). CTX, an inhibitor of the 1-EBIO activated K\(^{+}\) channels, decreased the I\(_{\text{SC}}\) to the pre-1-EBIO level. Panels B-E of Figure 5 show the Nyquist plots corresponding to each of the experimental conditions in Panel A. In this monolayer, the control impedance spectrum could be fit to the two membrane model shown in Figure 4 to yield estimates of R\(_{\text{ap}}\) and R\(_{\text{bl}}\) given in Panel 5B. Using these values one obtains an F\(_{\text{Rap}}\) of 0.53 in good agreement with the F\(_{\text{Rap}}\) of 0.52±0.26 obtained in the microelectrode studies. Stimulation with forskolin reduced the total impedance and two semicircles were clearly resolved in the Nyquist plot (Figure 5C). Based on morphological considerations and pharmacological studies the smaller semicircle closest to the origin can be identified as the apical membrane and the larger semicircle to the right as the basolateral membrane. Stimulation with forskolin reduced R\(_{\text{ap}}\) to 20 Ω cm\(^2\) and R\(_{\text{bl}}\) to 160 Ω cm\(^2\) to yield an F\(_{\text{Rap}}\) of 0.1, a value that is also in excellent agreement with the F\(_{\text{Rap}}\) of 0.08±0.005 obtained in the microelectrode studies. These results demonstrate that forskolin activates both apical membrane and basolateral membrane conductances. However, the change in the apical conductance far exceeds the change in the basolateral conductance and this likely explains why V\(_{\text{bl}}\) depolarizes in forskolin stimulated monolayers. The subsequent addition of 1-EBIO further reduced the total

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**Figure 4.** Equivalent electric circuit used to obtain estimates of the apical and basolateral membrane resistances (R) and capacitances (C) by impedance analysis. Two RC elements are shown connected in series and represent the apical and basolateral membranes, respectively. The RC elements are also connected in parallel with the paracellular pathway (PCP) shunt resistance (R\(_{\text{P}}\)) and a series resistance (R\(_{\text{S}}\)) for the solution resistance between the measuring electrodes and the cell monolayer.
impedance and caused a decrease in $R_{bl}$ to 77 Ω cm$^2$, consistent with the activation of basolateral membrane K$^+$ channels, and the repolarization of $V_{ap}$ and $V_{bl}$ observed in the microelectrode studies. One would also anticipate from the impedance results that $F_{Rap}$ should increase to 0.19 but this effect was not observed in the microelectrode studies. As expected for the blockade of the basolateral membrane 1-EBIO activated K$^+$ channels, CTX caused the total impedance to increase and increased $R_{bl}$ to 140 Ω cm$^2$, a value approaching the pre 1-EBIO $R_{bl}$ of 167 Ω cm$^2$.

The above impedance results demonstrate forskolin and 1-EBIO have their expected effects on the apical and basolateral membranes. The results shown in Figure 5 were selected from a large number of impedance experiments to illustrate the presence of both the apical and basolateral membranes components in the Nyquist plots. However, in most experiments the apical membrane could

![Diagram](image1)

**Figure 5.** Short circuit current trace from a Calu-3 monolayer showing the responses to forskolin, 1-EBIO and CTX along with the corresponding Nyquist plots under each of these conditions. Plotted are the real ($Z_R$) and imaginary ($Z_I$) components of the impedance at 100 sine wave frequencies over a range of 1 Hz to 22 kHz. The solid line is the fit obtained using the equations that describe the equivalent electric circuit shown in Figure 4. Values for $R_S$, $R_P$, $C_{ap}$ and $C_{bl}$ were 24 Ω cm$^2$, 310 Ω cm$^2$, 6 µF/cm$^2$ and 18 µF/cm$^2$, respectively.
not be discerned in the Nyquist plot once the cells were stimulated with maximal stimulatory concentrations of forskolin. Time course and dose response studies during the forskolin stimulated increase in \( I_{SC} \) revealed the presence of the apical membrane component (Figure 6). The decrease in the apical membrane impedance continued to a point where it essentially vanished from the Nyquist plot as \( I_{SC} \) continued to increase. If one uses data from the spectrum just prior to when the apical membrane vanishes, estimates of the apical membrane resistance in these monolayers falls below \( 5 \ \Omega \ \text{cm}^2 \). This is an astonishingly low value but nonetheless a value that is consistent with the very high levels of CFTR expressed by Calu-3 cells [11]. Thus, in most monolayers it was not possible by impedance analysis to obtain a true estimate of \( R_{ap} \) in the forskolin or forskolin plus 1-EBIO stimulated monolayers. Rather, \( R_{ap} \) would appear to be less than \( 5 \ \Omega \ \text{cm}^2 \) in the stimulated monolayers. This suggests that the apical membrane conductance (\( G_{ap} = 1/R_{ap} \)) is greater than 200 mS/cm\(^2\) in forskolin stimulated Calu-3 cells. If one uses the microelectrode derived estimate of \( F_{Rap} \) of \( 0.08 \pm 0.005 \) and the impedance derived estimate of \( R_{bl} \) of approximately 170 \( \Omega \ \text{cm}^2 \) an \( R_{ap} \) of approximately 14 \( \Omega \ \text{cm}^2 \) is calculated for forskolin stimulated Calu-3 cells. Therefore, a

**Figure 6.** Short circuit current trace and Nyquist plots at different concentrations of forskolin. A forskolin dose response was performed to obtain different levels of \( I_{SC} \) and impedance spectra were obtained at each new steady state. Impedance data were fit to the equations that describe the equivalent electric circuit shown in Figure 4. Note the vanishingly small portion of the impedance that can be attributed to the apical membrane as the \( I_{SC} \) increased.
conservative approximation of $G_{ap}$ in forskolin stimulated Calu-3 cells would be in the range of 50 to 200 mS/cm$^2$.

The astonishingly high $G_{ap}$ of forskolin stimulated Calu-3 cells has several important implications for anion secretion by these cells. We propose that this $G_{ap}$ is used to clamp the apical membrane at $E_{Cl}$ and thus insures a driving force for $HCO_3^-$ secretion equal to $V_{ap} - E_{HCO3}$ where $V_{ap} = E_{Cl}$ in forskolin stimulated cells. Based on our measured values for $V_{ap}$ and assuming an intracellular $HCO_3^-$ concentration of 15 mM (pH$_i$ = 7.15), there would be a driving force of 7.8 mV for the conductive exit of $HCO_3^-$ across the apical membrane. Patch clamp estimates of the $HCO_3^-$ to $Cl^-$ selectively of CFTR have yielded values in the 0.1 to 0.25 range [12, 13]. Machen and coworkers [14, 15] have obtained similar anion selectivity ratios in alpha toxin basolateral membrane permeabilized Calu-3 monolayers. Thus, if one does a calculation using the “worst case” estimates of a selectivity of 0.1 for $HCO_3^-$ to $Cl^-$ and $G_{ap}$ of 50 mS/cm$^2$ so that $G_{apHCO3} = 5$ mS/cm$^2$ ($G_{apHCO3} = 0.1 \times 50$ mS/cm$^2 = 5$ mS/cm$^2$) with a driving force of 7.8 mV, a $HCO_3^-$ current of approximately 40 $\mu$A/cm$^2$ is possible. On the hand if the selectivity were 0.25 and the $G_{ap}$ 200 mS/cm$^2$, ($G_{apHCO3} = 0.25 \times 200$ mS/cm$^2 = 50$ mS/cm$^2$) a current of 390 $\mu$A/cm$^2$ could be observed. The actual measured rate of $HCO_3^-$ secretion in forskolin stimulated Calu-3 cells is approximately 60 $\mu$A/cm$^2$ suggesting the actual $G_{apHCO3}$ will be closer to the lower value. Therefore, even though the conductance of CFTR is lower for $HCO_3^-$ compared to $Cl^-$ there is an adequate driving force to account for the secretion of $HCO_3^-$. Moreover, these results suggest that in cAMP stimulated Calu-3 cells CFTR serves as a $Cl^-$ conductance to set the driving force for another anion, $HCO_3^-$, and not for the secretion of $Cl^-$ as is commonly held in other models of $HCO_3^-$ secretion.

Why is $Cl^-$ not secreted by the forskolin stimulated Calu-3 cells? The reasons appear to be several fold. First, it would appear that the very high $G_{ap}$ dominates the total cellular conductance even though forskolin was observed to activate a basolateral membrane conductance. Thus, unlike other $Cl^-$ secretory cells the activation of this basolateral membrane conductance by cAMP is relatively small compared to the activation of $G_{ap}$ and does not adequately repolarize $V_{ap}$ to provide a driving force for $Cl^-$ exit across the apical membrane. Secondly, the $Na^+:K^+:2Cl^-$ cotransporter would appear to be inactive in forskolin stimulated Calu-3 cells [1]. Isotope flux studies failed to detect any bumetanide sensitive $Cl^-$ flux in forskolin treated Calu-3 cells. This is not because Calu-3 cells lack the $Na^+:K^+:2Cl^-$ cotransport because once stimulated by 1-EBIO the $I_{SC}$ becomes bumetanide sensitive and there is an increase in the serosal-to-mucosal flux of $Cl^-$ that is inhibited by bumetanide. Thus, only after 1-EBIO repolarizes $V_{ap}$ and $V_{bl}$ does the $Na^+:K^+:2Cl^-$ cotransporter become active. Based on studies in other systems, the signals that activate the $Na^+:K^+:2Cl^-$ cotransporter are a decrease in cell volume and a fall in the intracellular $Cl^-$ concentration [16]. The activation of basolateral membrane $K^+$ channels by 1-EBIO is expected to do both and studies are in progress to measure these changes. Based on the measured rate of $Cl^-$ secretion of approximately 130 $\mu$A/cm$^2$ and a $G_{ap}$ of 50 to 200 mS/cm$^2$ the necessary driving force to sustain this level of $Cl^-$ secretion is only 0.7 mV to 2.6 mV. If the intracellular $Cl^-$ activity were to remain unchanged when 1-EBIO was added, the repolarization of $V_{ap}$ by 5.9 mV would yield a current of 295 $\mu$A/cm$^2$ ($G_{ap} = 50$ mS/cm$^2$) to 1180 $\mu$A/cm$^2$ ($G_{ap} = 200$ mS/cm$^2$). Since the measured $I_{SC}$ is only 130 $\mu$A/cm$^2$, we predict that the intracellular $Cl^-$ activity will fall by approximately 8 mM thereby causing a shift in $E_{Cl}$ in keeping with the observed $I_{SC}$ and high $G_{ap}$.

In summary, the microelectrode and impedance results reported here lend additional support to
our proposed model of anion secretion in Calu-3 cells (Figure 1). The studies with Calu-3 cells establish an electrochemical profile against which results from submucosal gland serous cells can be compared to determine whether native serous cells secrete anions in a similar manner. If our results with Calu-3 cells are representative of airway serous cells, then HCO$_3^-$ secretion in the airways may be more important than has previously been appreciated. In addition, these studies and our proposed model for HCO$_3^-$ and Cl$^-$ secretion by the same cell may help explain the pathophysiology of anion secretion in the pancreas and small intestine of cystic fibrosis patients. If our model is correct, CFTR serves as the conductive pathway for HCO$_3^-$ exit across the apical membrane in HCO$_3^-$ secreting cells. Mutations in CFTR that impair the conductance of the channel for HCO$_3^-$ are expected to increase the severity of the disease in those epithelia where HCO$_3^-$ secretion is essential for the normal physiology of the organ. Impaired HCO$_3^-$ secretion in the pancreas and small intestine in cystic fibrosis patients has been known for many years. The results with Calu-3 cells suggest HCO$_3^-$ secretion may also be important in the airways.

**Key words** Bicarbonates; Chlorides; Secretions

**Abbreviations**
- $C_{ap}$: apical membrane capacitance
- $C_{bl}$: basolateral membrane capacitance
- CFTR: cystic fibrosis transmembrane conductance regulator
- CTX: charybdotoxin
- DNDS: 4,4’-dinitrostilben-2,2’-disulfonic acid
- $E_{Cl}$: equilibrium potential for Cl$^-$
- $E_{HCO3}$: equilibrium potential for HCO$_3^-$
- $E_{\text{RevNBC}}$: equilibrium reversal potential of the DNDS-sensitive NBC
- $F_{ap}$: apical membrane fractional resistance
- $G_{ap}$: apical membrane conductance
- $G_T$: transepithelial conductance
- $K_{Ca}$: Ca$^{2+}$-activated, charybdotoxin-sensitive K$^+$ channels
- NBC: sodium bicarbonate cotransporter
- PKA: protein kinase A
- $R_{ap}$: apical membrane resistance
- $R_{bl}$: basolateral membrane resistance
- $R_F$: resistance of the paracellular pathway
- $R_T$: transepithelial resistance
- $V_{ap}$: apical membrane potential
- $V_{bl}$: basolateral membrane potential
- $V_T$: transepithelial potential
- $Z_i$: imaginary impedance
- $Z_R$: real impedance

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