CFTR and Bicarbonate Secretion to Epithelial Cells

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Defective HCO3− and fluid secretion are hallmarks of the pathophysiology of the pancreas of cystic fibrosis patients. Recently, impaired HCO3− secretion has been shown in most tissues known to express the cystic fibrosis transmembrane conductance regulator (CFTR). New results suggest that CFTR plays an important role in the transcellular secretion of HCO3−.

The cystic fibrosis transmembrane conductance regulator (CFTR) plays a crucial role in maintaining fluid secretion of epithelial cells of the airways and the intestine. Defective CFTR leads to an imbalance between fluid absorption and secretion in the lungs of cystic fibrosis (CF) patients, resulting in a relatively dehydrated mucus layer on the airways. However, the onset of clear symptoms of impaired lung function remains highly variable. A striking contrast can be found when one examines the exocrine pancreas. Among all CF patients, 70–90% are born with pancreatic insufficiency, which means that >98% of the pancreatic capacity is already lost (17). Even in the seemingly pancreatic-sufficient patients, the ratio between alkaline fluid and secreted digestive enzymes is significantly decreased (8). Clinicians have been using the amount of residual pancreatic function to classify CF patients into severe and mild cases. Under physiological conditions, the secreted HCO3−-rich fluid and electrolytes serve to flush the pancreatic tissue. In most of the pancreatic-insufficient patients, the tissue damage has already taken place in utero; however, in some cases the process may develop over a period of many years. Pancreatic insufficiency leads to malabsorption and severe steatorrhea, with concomitant losses of lipid-soluble vitamins and essential fatty acids. The malabsorption renders the patients more susceptible to infections, also aggravating the lung symptoms of the patients. Fortunately, the deficiencies of pancreatic insufficiency can be compensated in large measure by dietary supplementation.

The exocrine pancreas consists of two morphologically distinct structures: 1) acinar cells that secrete enzymes, mucins, and NaCl and 2) duct cells that mainly secrete a HCO3−-rich fluid. The cause for the pancreatic insufficiency in CF has been attributed to a lack of ductal function, whereas the acinar cells show only small or no abnormalities at all. Thus it was no surprise that immunocytochemical studies localized CFTR almost exclusively to the pancreatic ductal cells, although a number of reports demonstrated some scanty expression in the acinar cells. The general concept of ductal fluid secretion has been developed over the past 60 years. Bro-Rasmussen and colleagues (1) were among the first to correlate the HCO3− concentration with the secretory output of the pancreas after stimulation with the hormone secretin, a cAMP-mediated agonist (1). The observed inverse correlation between the HCO3− and the Cl− content of the pancreatic juice can now be found in most textbooks of physiology. In a series of elegant experiments on perfused pancreatic ducts (13), Ivana Novak and Rainer Greger demonstrated that the ductal epithelial cell has a unique mechanism for its secretory function. The simple model in Fig. 1 depicts their basic hypothesis for ductal HCO3− secretion.

HCO3− ions are generated from CO2 that enters the cell from the basolateral side by passive diffusion. The activity of carbonic anhydrase in the duct cell catalyzes the formation of carbonic acid from CO2 and H2O and the subsequent dissociation into HCO3− and protons. The latter are extruded through the basolateral membrane via a secondary active Na+/H+ exchanger. The driving force for the antiporter is provided by the Na+ pump, establishing the concentration gradient for Na+. Basolateral K+ channels maintain a hyperpolarized basolateral membrane. For a number of species, an additional Na+-dependent HCO3− uptake mechanism on the basolateral mem-


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brane of pancreatic duct cells has been demonstrated. The HCO₃⁻ ions that are accumulated by these mechanisms leave the cell on the apical membrane via a disulfonic stilbene-sensitive pathway in exchange for Cl⁻. To this end, the molecular identity of this anion exchanger (AE) in the pancreatic duct has not yet been identified. The classic AE1 that was first identified in the red blood cell and the other members of this family (AE2 and AE3) are not expressed on the apical membrane of HCO₃⁻-secreting epithelial cells. Other proteins like downregulated in adenoma (DRA; SLC26A3), (19) pendrin (SLC26A4), and a member of the putative anion transporter family (PAT1; SLC26A6) have been implicated as likely candidates for this mechanism (12). The Cl⁻ ions required for the exchange process are provided by the Cl⁻-rich acinar fluid and are recycled via luminal Cl⁻ channels. The concerted actions of apical Cl⁻ channels and basolateral K⁺ channels create a lumen-negative transepithelial voltage that draws Na⁺ and H₂O across the epithelium into the lumen. The proposed mechanism might explain the earlier finding that with increasing HCO₃⁻ secretion the concentration of Cl⁻ decreases. It also highlights the crucial role of CFTR in this mechanism. The seeming void of any other Cl⁻ export mechanisms ties the function of the AE inseparably to the only Cl⁻ channel detected in the apical membrane of this epithelium, which is considered to be CFTR. A defect in the luminal Cl⁻ conductance would eventually abolish both HCO₃⁻ and fluid secretion.

Does CFTR directly regulate anion exchange?

The coupling between HCO₃⁻ transport and CFTR has recently been reinvestigated (10), and it was postulated that the activity of the putative AE per se is regulated by CFTR and is not dependent on Cl⁻ movement through the channel. On the basis of the observation that some mutations in the CFTR gene render CF patients pancreatic sufficient but most others do not, Choi and coworkers (2) expressed selected CFTR mutations in fibroblasts. In the subsequent functional studies, the authors recognized that some of the mutations showed an apparent lack in their HCO₃⁻ transport, as assessed by the rate of alkalization in Cl⁻-free medium, whereas the Cl⁻ transport (detected by a Cl⁻-sensitive fluorophore) seemed to be unaffected and vice versa. The comparison of the results in the expression system with clinical data yielded an interesting correlation. Mutations that led to impaired HCO₃⁻ transport in the expression system were only found in the pancreatic-insufficient patients, and those mutations that solely produced a decreased Cl⁻ conductance were found in pancreatic-sufficient patients. The authors proposed that the impaired HCO₃⁻ transport resulted from a disruption between CFTR and the anion exchange mechanism and therefore could be held responsible for the fatal pathogenesis in CF in all tissues expressing both proteins. This work has drawn quite a bit of attention toward the field of HCO₃⁻ transport. Nevertheless, the results of this study should be regarded with caution. Some carriers of mutations that were seemingly associated with an intact Cl⁻ conductance did have abnormal sweat Cl⁻ concentrations, indicative of an impaired Cl⁻ permeability in the sweat duct (20). Moreover, whereas the coupled AE/Cl⁻ con-

![FIGURE 1. HCO₃ secretion by the rat pancreatic duct cell. The lipid-permeable CO₂ enters the cell through the basolateral membrane and serves as a pool for the generation of H₂HCO₃ and, subsequently, HCO₃. HCO₃ leaves the cell via a luminal anion exchanger. The accumulated Cl⁻ recycles via the luminal Cl⁻ channels.](https://example.com/image.png)
impaired in CF cells (3, 18). In non-CF cells, amiloride causes a 50–70% decrease in the short-circuit current (Isc). The residual Isc requires HCO3− and not Cl− in the bathing solution and is partially inhibited by serosal DNDS, a disulfonic stilbene that blocks HCO3− transporters. cAMP causes a further increase in the Isc, and this increase requires HCO3− in the bathing solution and is inhibited by serosal DNDS. Thus non-CF cells display a basal level of HCO3− secretion, and this can be stimulated by cAMP. In CF cells, amiloride inhibits nearly all of the Isc, and cAMP fails to cause an increase in the Isc. Studies of this nature led Smith and Welsh (18) to conclude that HCO3− secretion is impaired in CF airway epithelia and that HCO3− exit at the apical membrane is through the anion channel that is defectively regulated in CF epithelia (18).

Secretion of airway serous cells: lessons from Calu-3 cell studies

Airway epithelia can be divided in two different functional entities: primarily absorptive cells and secretory cells. The absorptive surface epithelia of the airways express high levels of the epithelial Na+ channel (ENaC), whereas the CFTR expression is rather scanty. In contrast, the secretory serous cells of the submucosal glands lack ENaC expression and have been demonstrated to be the predominant site of CFTR expression in the airways, expressing manyfold higher levels of CFTR compared with the surface airway epithelium. Recent studies on an airway serous cell line, Calu-3, have provided further support that airway cells secrete HCO3− in response to cAMP (4, 9). Calu-3 cells resemble the characteristics of airway serous cells and can be grown as polarized monolayers for transport studies. Thus the Calu-3 cells have served as a model for airway serous cells, and studies with the Calu-3 cells have provided important insight into the underlying mechanisms of HCO3− secretion.

Calu-3 cells display a low basal Isc that increases upon stimulation with forskolin. Isotope flux studies revealed that the forskolin-stimulated Isc was not the result of net CI−, Na+, or K+ transport, leaving HCO3− secretion as the likely basis of the ion substitution studies showed that the forskolin-stimulated secretion did not require Cl− in the mucosal or serosal bathing solutions but did require serosal HCO3− and serosal Na+. Bumetanide, an inhibitor of the Na+/K+−2Cl− cotransporter, also failed to block the forskolin-stimulated Isc. In contrast, serosal DNDS, but not mucosal DNDS, partially inhibited the forskolin-stimulated Isc. These results led us to conclude that Calu-3 cells secrete HCO3− by an electrogenic mechanism in response to forskolin stimulation. We proposed that HCO3− influx across the basolateral membrane was mediated by a DNDS-sensitive Na+/HCO3− cotransporter (NBC). HCO3− exit across the apical membrane did not require luminal Cl−, nor was it inhibited by mucosal DNDS. Thus we proposed that HCO3− exit was mediated by CFTR.

The switch from HCO3− to Cl− secretion: driving force matters

An important feature of anion secretion by the Calu-3 cells was revealed by the use of 1-ethyl benzimidazolone (1-EBIO). 1-EBIO activates Ca2+-activated K+ channels such as hIK-1, unlike with agonists like acetylcholine the activation of these channels is prolonged. The addition of 1-EBIO to forskolin-stimulated Calu-3 cells caused a further increase in the Isc, which was expected for the activation of basolateral membrane K+ channels and the hyperpolarization of the membrane potential. However, in contrast to stimulation with forskolin alone, stimulation with forskolin plus 1-EBIO caused the net secretion of Cl−, as revealed by isotope flux studies and the inhibition of the Isc by bumetanide. Indeed, the Isc of forskolin plus 1-EBIO-stimulated cells was fully accounted for by the net secretion of Cl−. Figure 3 illustrates these findings.

![FIGURE 2. HCO3− secretion by distal pancreatic ducts. The decrease in the luminal Cl− concentration leads to a subsequent loss of driving force for the anion exchange mechanism. To sustain further Cl−-independent HCO3− secretion, an alternative HCO3− exit via an electrogenic pathway was postulated. The n (in nHCO3−) refers to the number of ions transported with a single Na+ ion and varies between 2 and 3.](image-url)
The activation of basolateral K+ channels caused Calu-3 cell anion secretion to switch from HCO3− secretion to Cl− secretion. These results led us to propose that the basolateral membrane NBC was an electrogenic transporter carrying a greater number of HCO3− anions than Na+ cations. Hyperpolarization of the basolateral membrane potential as a result of the activation of K+ channels by 1-EBIO would tend to inhibit HCO3− influx across the basolateral membrane. Indeed, if the basolateral membrane potential were to exceed the reversal potential of the NBC, HCO3− might exit rather than enter the cell across the basolateral membrane. Concomitant with the inhibition of an electrogenic NBC, the Na+-K'−2Cl− cotransporter is activated in forskolin plus 1-EBIO-stimulated cells.

Consistent with the very high levels of CFTR expression, forskolin causes the apical membrane resistance to fall to a remarkably low value. At the same time we observe a depolarization of the apical membrane potential to the equilibrium potential for Cl−. This effect is so dominant that it also depolarizes the basolateral membrane due to the low shunt resistance of the paracellular pathway. The activation of basolateral membrane K+ channels by forskolin, as is evident from the decrease in the basolateral membrane resistance upon forskolin stimulation, is insufficient to maintain the basolateral membrane potential.

Instead the basolateral membrane depolarizes, and this provides a favorable membrane potential for HCO3− entry on an electrogenic NBC. Whether forskolin also activates the NBC via PKA-mediated phosphorylation is not known at this time. Thus the very high apical membrane anion conductance stimulated by forskolin 1) serves to mediate the conductive exit of HCO3−, 2) sets the driving force for HCO3− exit across the apical membrane, and 3) sets the driving force for the entry of HCO3− across the basolateral membrane on the NBC.

As expected for the activation of basolateral membrane K+ channels by 1-EBIO, the basolateral membrane and apical membrane potentials are seen to hyperpolarize from the forskolin-stimulated values. The hyperpolarization of the apical membrane would be expected to increase the driving force for both HCO3− and Cl− exit and thus cannot explain the inhibition of HCO3− secretion. However, the hyperpolarization of the basolateral membrane potential is expected to inhibit the influx of HCO3− mediated by an electrogenic NBC that moves a net anionic charge. The removal of Na+ or HCO3− from the serosal solution or the addition of DNDS both result in the depolarization of the basolateral membrane potential as expected for an electrogenic NBC (Tamada, Hug, and Bridges, unpublished observations). Indeed, one may deduce...
from the basolateral membrane potential measurements in forskolin and forskolin plus 1-EBIO-stimulated cells that the Na\(^+\)-HCO\(_3\) stoichiometry of the NBC in Calu-3 cells is 1:3. On the basis of these observations, we proposed a model for anion secretion by airway serous cells that is depicted in Fig. 4.

**Summary**

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 CF patients. If our model is correct, CFTR serves as the conductive pathway for HCO\(_3\)\(^-\) in the normal physiology of the organ. Impaired HCO\(_3\)\(^-\) secretion in the pancreas and small intestine in CF patients has been known for many years. The results with primary cultures of human bronchial epithelial cells and Calu-3 cells suggest that HCO\(_3\)\(^-\) secretion may also be important in the airways. It seems prudent to speculate that a similar mechanism to that found in Calu-3 cells might be attributable to other epithelia that secrete HCO\(_3\)\(^-\).

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**References**