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MINI-REVIEW

The role of CFTR in bicarbonate secretion by pancreatic duct and airway epithelia

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Abstract : The secretory epithelia of the pancreatic duct and airway share the ability to generate HCO_3^--rich fluids. They both express CFTR (cystic fibrosis transmembrane conductance regulator) at the apical membrane and both are adversely affected by cystic fibrosis. CFTR is predominantly a Cl^- channel, and it is widely believed that HCO_3^- secretion in the pancreatic duct is mediated mainly by a Cl^-/HCO_3^- exchanger at the apical membrane. Studies on airway epithelia, however, have suggested that CFTR, despite its low permeability to HCO_3^-, may nonetheless be directly responsible for HCO_3^- secretion across the apical membrane. This article reviews recent work that has re-examined both of these hypotheses. J. Med. Invest. 56 Suppl. : 336-342, December, 2009

INTRODUCTION

Many exocrine glands secrete an isotonic, Na^+- and Cl^--rich primary secretion, which is modified as it passes through the gland's ductal system. However, there are a number of physiologically important epithelia that secrete a HCO_3^--rich primary fluid. Examples include the secretory epithelia of the pancreatic duct, duodenum and airways. In each of these, the cystic fibrosis transmembrane conductance regulator (CFTR) appears to play a major role. This is perhaps surprising because CFTR functions primarily as a Cl^- ion channel and has only a low permeability to HCO_3^- ions (1-3). On the other hand, many of the symptoms of cystic fibrosis can be attributed to a lack of HCO_3^- secretion (4). It has therefore become important to clarify the precise role of CFTR in the secretion of HCO_3^- ions.

BASOLATERAL HCO_3^- UPTAKE

The mechanism of HCO_3^- uptake across the basolateral membrane of secretory epithelia is now relatively well understood. There are two fundamentally distinct pathways (Fig. 1) whose relative
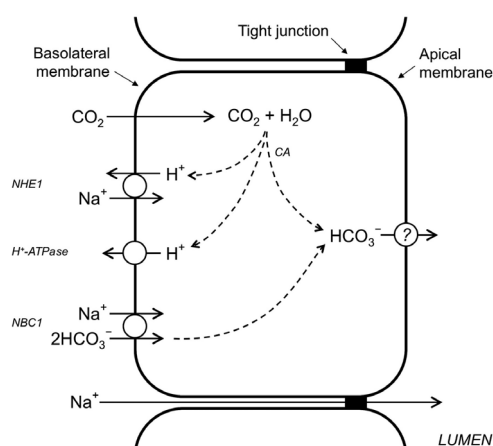

Fig. 1   Pathways for HCO_3^- accumulation across the basolateral membrane of secretory epithelia. Hydration of CO_2 entering the cell by diffusion is facilitated by carbonic anhydrase (CA) and provides a source of intracellular HCO_3^-. Protons are extruded across the basolateral membrane either by Na^+/H^+ exchange (NHE1) or *via* a vesicular-type H^+-ATPase. HCO_3^- may also be brought into the cell *via* Na^+-HCO_3^- cotransporter (NBC1).

contributions vary between different tissues and between resting and secreting states. One involves the diffusion of CO_2 into the cell, its hydration and dissociation to H^+ and HCO_3^- ions under the influence of carbonic anhydrase, and the subsequent extrusion of H^+ ions across the basolateral membrane, either by Na^+/H^+ exchange or via a vesicular-type H^+-ATPase. The alternative pathway involves the direct uptake of HCO_3^- by a Na^+-HCO_3^- cotransporter, usually the pancreatic splice variant of NBC1 (pNBC1 or NBCe1-B).

These basolateral transporters also have a 'housekeeping' role in the regulation of intracellular pH (pH_i), which is maintained at a value close to, or slightly below, the extracellular pH. The regulation of pH_i at ~7.2 ensures that the intracellular HCO_3^- concentration is ~20 mM. Together with a membrane potential of around -60 mV, which is determined mainly by the K^+ concentration gradient across the basolateral membrane, this means that there is an outwardly directed electrochemical gradient for HCO_3^-.

It has been known for some time that inhibitors of carbonic anhydrase may have only a modest effect on epithelial HCO_3^- secretion, so it was no surprise to discover that, during maximal secretion in the pancreatic duct, much of the secreted HCO_3^- enters the cells via the basolateral NBC1 rather than as CO_2 (5). Because this transporter is electrogenic, carrying two HCO_3^- ions with each Na^+ ion, its activity also helps to maintain the membrane potential by counterbalancing the depolarizing effect of Cl^- and HCO_3^- efflux across the apical membrane. The leakiness of the tight junctions to cations in these epithelia ensures that the apical and basolateral membrane potentials are always within a few millivolts of each other. This electrical coupling helps to match the rates of HCO_3^- entry and exit so that pH_i is not adversely affected by changes in secretory rate.

In summary, therefore, the basolateral mechanisms responsible for pH_i regulation and the maintenance of the membrane potential ensure that there is normally an outwardly directed electrochemical gradient for HCO_3^- at the apical membrane that could theoretically drive HCO_3^- efflux into a HCO_3^--rich secretion. However, the nature of apical efflux pathway has been difficult to establish with any certainty. There are again two distinct possibilities (Fig. 2). One is that HCO_3^-, like Cl^-, leaves the cell by electrodiffusion through CFTR or some other anion channel in the apical membrane. The alternative
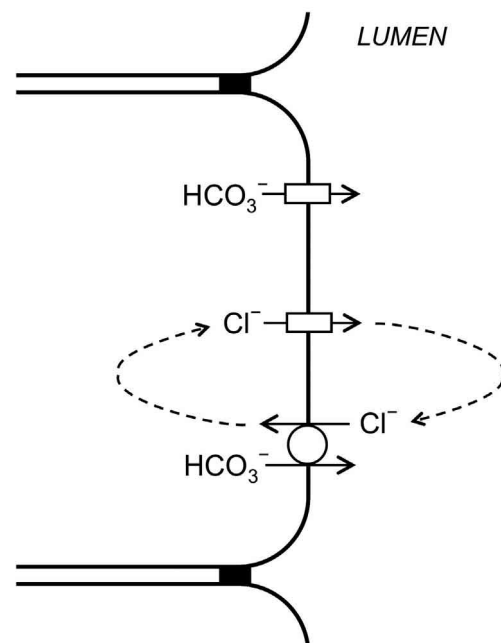

Fig. 2   Alternative mechanisms proposed for HCO_3^- secretion across the apical membrane of secretory epithelia. HCO_3^- may either leave the cell by electrodiffusion through a ion channel (top) or by exchange with Cl^- (middle). In the latter case, Cl^- is supplied to the lumen *via* an ion channel (bottom) and it re-enters the cell in exchange for HCO_3^-.

is that HCO_3^- is secreted via an anion exchanger, most probably a Cl^-/HCO_3^- exchanger. We now consider the evidence for each of these in turn.

APICAL Cl^-/HCO_3^- EXCHANGERS

From some of the earliest studies on isolated pancreatic ducts (6, 7) it was clear that a Cl^-/HCO_3^- exchanger was present at the apical membrane of pancreatic duct epithelium. At that time, the known Cl^-/HCO_3^- exchangers of the AE (SLC4) family had been shown to mediate 1 : 1 exchange of Cl^- and HCO_3^- ions, and were therefore electrically neutral. However, ductal secretion evoked by secretin was known to establish a negative electrical potential in the duct lumen, so it was proposed that the Cl^-/HCO_3^- exchanger operates in parallel with a cAMP-regulated Cl^- channel. This was subsequently identified as CFTR (8). The net effect would be that HCO_3^- is secreted into the lumen in exchange for luminal Cl^-, and the supply of Cl^- to the lumen is maintained by electrogenic Cl^- efflux *via* CFTR.

This model accounts well for the secretion of

moderately HCO_3^--rich fluid by the rat pancreas where the maximum HCO_3^- concentration is about 75 mM. However, the model struggles to account for the much higher HCO_3^- concentrations (up to 140 mM) that are observed in other species such as the guinea-pig and human (9, 10). The main difficulty is that, with 140 mM HCO_3^- and 20 mM Cl^- in the lumen, an apical Cl^-/HCO_3^- exchanger with a 1 : 1 stoichiometry would reverse and reabsorb HCO_3^- rather than secrete it (Fig. 3) (11).

For many years the molecular identity of the apical Cl^-/HCO_3^- exchanger in the pancreatic duct remained unknown but it now seems likely that it is a member of the SLC26 family of versatile anion transporters, specifically SLC26A6 (12). Furthermore, there is now strong evidence that this exchanger is electrogenic and that it mediates the exchange of one Cl^- ion for two HCO_3^- ions (13) although there may be differences in stoichiometry between species (14). At first, it was supposed that the 1 : 2 stoichiometry might enable the exchanger to secrete HCO_3^- ions against a steeper concentration gradient than a 1 : 1 exchanger. Calculations showed, however, that in the guinea-pig pancreatic duct the exchanger will reverse when the luminal

HCO_3^- concentration exceeds ~136 mM (Fig. 3) (10). In other words, the exchanger cannot provide the main pathway for HCO_3^- secretion at a concentration of 140 mM HCO_3^- because it will be very close to equilibrium under those conditions. The main advantage of the 1 : 2 stoichiometry could simply be that it prevents significant reabsorption of HCO_3^-. It is also clear that, during the initial stages of secretion, and in duct segments close to the pancreatic acini, which secrete small amounts of Cl^--rich fluid, the SLC26A6 exchanger will play a significant role in raising the luminal HCO_3^- concentration and reabsorbing luminal Cl^-. But in the more distal ducts, once secretion is established, the exchanger cannot be the main pathway for HCO_3^- secretion.

CFTR AS A HCO_3^- CHANNEL

The only alternative mechanism that has been proposed to account for HCO_3^- secretion under these conditions is the passive efflux of HCO_3^- down its electrochemical gradient through an ion channel. Because the intracellular HCO_3^- concentration is clamped by pH_i regulation at ~20 mM and the membrane potential (apical and basolateral) is maintained at around -60 mV, there is normally a steep electrochemical gradient for HCO_3^- efflux from the cell. Inserting these figures in the Nernst equation tells us that HCO_3^- efflux would only cease if the luminal HCO_3^- concentration reached or exceeded ~200 mM, which means that 140 mM could, in theory, be attained quite easily by this mechanism.

The problem with this model is that the only known cAMP-activated anion channel in the apical membrane of pancreatic duct cells is CFTR, and the HCO_3^- permeability of CFTR is only ~25% of its permeability to Cl^-. However, there have been several studies suggesting that the relative permeability of CFTR to HCO_3^- is variable (15) and may increase when the extracellular Cl^- concentration is low (16). On the other hand, another recent study has shown that the permeability ratio does not change under these conditions (17).

Attempts to measure the total HCO_3^- permeability of the CFTR channels in the apical membrane of the guinea-pig pancreatic duct have indicated that it is probably sufficient to account for at least one half of the secreted HCO_3^-, possibly more (or less) given the many uncertainties in the calculation (18). So, regardless of whether the HCO_3^- permeability of CFTR is modulated, it seems likely that CFTR
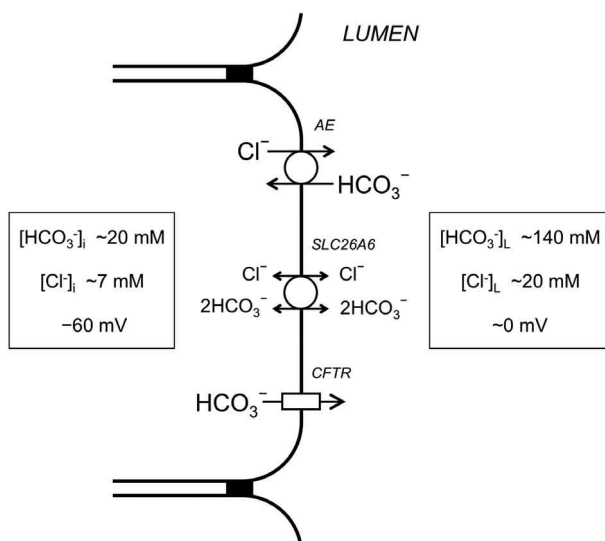

Fig. 3 Predicted direction of HCO_3^- transport through alternative apical membrane transporters in the guinea-pig pancreatic duct. Measured values for intracellular Cl^- concentration, pH_i and membrane potential are shown on the left. Observed concentrations of Cl^- and HCO_3^- in the secreted fluid during maximal stimulation are shown on the right. Under these conditions, a 1 : 1 Cl^-/HCO_3^- exchanger (AE) would mediate HCO_3^- absorption and a 1 : 2 Cl^-/HCO_3^- exchanger (SLC26A6) would be approximately at equilibrium (10). In contrast, the driving force for HCO_3^- efflux *via* CFTR would still be in an outward direction, thus favouring secretion.

makes a significant contribution to the secretion of HCO_3^- at high concentrations. Whether the remainder can be explained by $Cl^-/2HCO_3^-$ exchange *via* SLC26A6 operating close to its equilibrium condition remains to be seen. Studies of SLC26A6 knockout mice in two different laboratories (19, 20) have yielded conflicting results in this regard, and it is unfortunate that the wild-type mouse pancreas secretes HCO_3^- at much lower concentrations than the guinea-pig and human and is therefore not a good experimental model.

Another factor that enables CFTR to provide a major pathway for HCO_3^- secretion in the pancreatic duct has emerged from studies comparing rat and guinea-pig ducts (21). One way of achieving a HCO_3^--rich secretion, despite the low relative permeability of CFTR to HCO_3^-, would be for the duct cell to generate a much larger electrochemical gradient for HCO_3^- than for Cl^-. We have seen that the HCO_3^- gradient is largely fixed by pH_i regulation, but it is clear that in the guinea-pig the gradient for Cl^- efflux *via* CFTR at the apical membrane drops to a low level during secretion (22). This seems to be because the basolateral membrane has only a limited capacity for Cl^- uptake in the guinea-pig. In contrast, rat ducts have Na^+-K^+-$2Cl^-$ cotransporters at the basolateral membrane which sustain Cl^- uptake during secretion. This Cl^- competes with HCO_3^- for efflux *via* CFTR at the apical membrane giving a mixed secretion of Cl^- and HCO_3^- (21). In other words, it may be the lack of Cl^- uptake at the basolateral membrane that leads to the secretion of a HCO_3^--rich fluid in the guinea-pig and human, rather than any special properties of the apical membrane.

HCO_3^- SECRETION BY AIRWAY EPITHELIUM

The idea that the anion composition of the secreted fluid might be determined mainly by the driving forces that are established for Cl^- and HCO_3^- at the basolateral membrane first arose in studies of Calu-3 airway cells (23). This cell line is derived from a human lung carcinoma and it shows many of the characteristics of the fluid-secreting serous cells that are found in the submucosal glands of the airways. Calu-3 cells express abundant CFTR and can secrete fluid containing up to 80 mM HCO_3^- in response to elevated cAMP (24). Perhaps surprisingly, given what we know about the pancreatic duct and also the duodenum (where CFTR and the SLC26A3 Cl^-/HCO_3^- exchanger work in tandem), previous studies on Calu-3 cells, mainly using the Ussing chamber technique, have concluded that both Cl^- and HCO_3^- leave the cell exclusively *via* the CFTR channels at the apical membrane (25). Although a Cl^-/HCO_3^- exchanger (AE2) has been identified at the basolateral membrane (26), no evidence has been reported for the existence of an exchanger at the apical membrane. This disparity between HCO_3^--secreting epithelia that otherwise share so many common features has led us to re-examine the possibility that an apical Cl^-/HCO_3^- exchanger might be present in Calu-3 cells and might therefore contribute to the secretion of HCO_3^-.

A standard protocol for detecting Cl^-/HCO_3^- exchange is to examine the changes in pH_i that result from substitution of extracellular Cl^- with a non-transported anion such as gluconate (Fig. 4). Under normal conditions, the ion gradients are such that
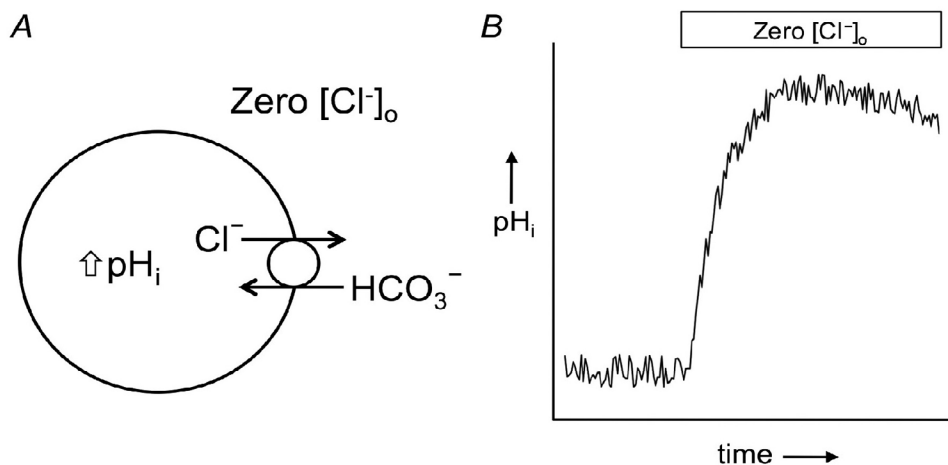

Fig. 4   Experimental protocol for detecting Cl^-/HCO_3^- exchanger activity. When extracellular Cl^- is replaced by an impermeant anion such as gluconate, the Cl^-/HCO_3^- exchanger reverses (A) and HCO_3^- entry leads to a rise in intracellular pH (B).

a Cl^-/HCO_3^- exchanger mediates an efflux of HCO_3^- and an influx of Cl^-. Reducing the extracellular Cl^- concentration to zero results in a swift reversal of the exchanger, which now brings HCO_3^- into the cell as Cl^- leaves down its steep concentration gradient. The entry of HCO_3^- leads to a rise in pH_i which, if mediated by an anion exchanger, should be blockable by an appropriate inhibitor such as DIDS.

In our studies (Kim, Best & Steward, unpublished data) we were unable to detect Cl^-/HCO_3^- exchange at the apical membrane of unstimulated Calu-3 cells. But, when the cells were stimulated with forskolin to elevate intracellular cAMP, there was a marked increase in pH_i in response to apical Cl^- substitution, suggesting that stimulation does indeed activate an exchanger at the apical membrane. Restoring Cl^- to the apical bath led to a rapid recovery of pH_i and this recovery could be induced by other monovalent anions, such as iodide and formate, but not by divalent anions such as sulfate and oxalate. Surprisingly, however, the apical exchanger appeared to be relatively insensitive to DIDS. These observations led us to review the anion selectivities and inhibitor sensitivities of the various members of the SLC26 family and we suggested that SCL26A4 (pendrin), which appears to be selective for monovalent anions and insensitive to DIDS, was the most likely candidate (27).

Subsequent studies, however, have cast doubt on this hypothesis. Firstly, an evaluation of mRNA expression levels for members of the SLC26 family only revealed low levels of expression of SLC26A2 and -A6, both of which, unlike our hypothetical exchanger, are known not to transport iodide. Using quantitative PCR we found that, compared with the plentiful expression of CFTR mRNA, the other Cl^-/HCO_3^- transporters of the SLC26 family (SLC26A3 and -A4) were barely detectable, whereas mRNA for the AE2 exchanger, known to be present at the basolateral membrane, was even more abundant than for CFTR.

The next problem with our hypothesis was that the alkalinization evoked by apical Cl^- substitution was almost completely abolished by the specific CFTR channel blocker, $CFTR_{inh}$-172. This suggested that CFTR, rather than an exchanger, might be responsible for the influx of HCO_3^- through the apical membrane. Certainly the efflux of Cl^- through CFTR would have the effect of depolarizing the cells and this, if large enough, could lead to a reversal of the electrochemical gradient for HCO_3^-. Using the Nernst equation, we estimated that the membrane

potential would have to reverse to a positive value of about +30 mV in order to drive sufficient HCO_3^- entry across the apical membrane to raise pH_i to the values that are observed in the experiments (~7.8). Although such a large change in membrane potential might seem unlikely, patch-clamp measurements on Calu-3 cells, using the perforated-patch configuration with gramicidin D as the ionophore, indicate that the membrane potential does reverse to positive values when forskolin-stimulated cells are exposed to Cl^--free solutions. Because the technique requires the isolation of single cells, the experimental conditions do not exactly replicate what occurs in the polarized epithelium. However, the results do lend some support to the idea that what appears to be HCO_3^- entry *via* an anion exchanger is in reality the entry of HCO_3^- through CFTR, and that this is driven by the membrane potential change evoked by Cl^- efflux. It also confirms previous studies suggesting (i) that Calu-3 cells probably do not express Cl^-/HCO_3^- exchangers at their apical membrane and (ii) that HCO_3^- secretion is mediated directly by CFTR.

CONCLUSIONS

From our work on Calu-3 cells, we conclude that experimental protocols designed to test for Cl^-/HCO_3^- exchanger activity also detect the exchange of Cl^- and HCO_3^- by electrodiffusion through ion channels. It is therefore essential to examine the effects of exchanger and channel inhibitors, and preferably also to measure mRNA and/or protein expression, before drawing any conclusions about the presence or absence of Cl^-/HCO_3^- exchangers.

Our second conclusion is that some epithelia are able to secrete HCO_3^--rich fluids with only CFTR providing the HCO_3^- efflux pathway at the apical membrane. This does not seem to require the involvement of a parallel anion exchanger to compensate for the low HCO_3^- permeability of CFTR, it simply requires that the driving force for Cl^- is held at a much lower level than that for HCO_3^-. Since the driving force for HCO_3^- is fixed by pH_i regulation, the relative sizes of the two driving forces, and therefore the composition of the secretion, depend largely on the activity of any Cl^- transporters that are present at the basolateral membrane.

ACKNOWLEDGEMENTS

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