Functional Interactions of HCO₃⁻ with Cystic Fibrosis Transmembrane Conductance Regulator

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Summary

Disruption of normal cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl⁻ transport is associated with cystic fibrosis (CF). CFTR is also required for HCO₃⁻ transport in many tissues such as the lungs, gastro-intestinal tract, and pancreas, although the exact role CFTR plays is uncertain. Given the importance of CFTR in HCO₃⁻ transport by so many CF-affected organ systems, it is perhaps surprising that relatively little is known about the interactions of HCO₃⁻ ions with CFTR. We have used patch clamp recordings from native pancreatic duct cells to study HCO₃⁻ permeation and interaction with CFTR. Ion selectivity studies shows that CFTR is between 3-5 times more selective for Cl⁻ over HCO₃⁻. In addition, extracellular HCO₃⁻ has a novel inhibitory effect on cAMP-stimulated CFTR currents carried by Cl⁻. The block by HCO₃⁻ was rapid, relatively independent of voltage and occurred over the physiological range of HCO₃⁻ concentrations. These data show that luminal HCO₃⁻ acts as a potent regulator of CFTR, and suggests that inhibition involves an external anion-binding site on the channel. This work has implications not only for elucidating mechanisms of HCO₃⁻ transport in epithelia, but also for approaches used to treat CF.

It is well established that cystic fibrosis transmembrane conductance regulator (CFTR) transports chloride ions in a variety of epithelial tissues. Disruption of normal CFTR-mediated Cl⁻ transport is associated with a number of diseases such as cystic fibrosis (CF), certain types of secretory diarrhoea, and possibly polycystic kidney disease. CFTR is also involved in the transport of other physiologically important anions such as HCO₃⁻ [1], glutathione [2] and larger organic anions [3]. In the case of HCO₃⁻ many epithelial tissues secrete this anion by a mechanism which is dependent on functional CFTR channels. This has been observed in the airways [4], including submucosal glands [5]; the gastro-intestinal tract [6]; the liver and gallbladder [7, 8] and the pancreas [9], the archetypal bicarbonate-transporting gland. While there is now strong evidence that CFTR is essential for effective HCO₃⁻ secretion the exact role it plays is still uncertain. Our studies have focused on the role of CFTR in the production of an HCO₃⁻ rich alkaline secretion by the exocrine pancreas [1]. We
proposed back in 1988 that HCO₃⁻ exits across the apical membrane of pancreatic duct cells (PDCs) by parallel operation of CFTR Cl⁻ channels and Cl⁻/HCO₃⁻ exchangers [10]. In this scheme the CFTR channel can be viewed as having two functions. The first is to provide luminal Cl⁻ for operation of the anion exchangers. The second is to act as a leak pathway to dissipate intracellular Cl⁻ accumulated as the exchanger cycle. Implicit in this ‘CFTR-anion exchanger model’ is that CFTR is better at transporting Cl⁻ than HCO₃⁻ under normal physiological conditions. We showed this to be the case in subsequent patch clamp studies using both single channel [11] and whole cell current recordings [12], of CFTR in native rat pancreatic duct cells. However, it should be noted that in all cases CFTR did demonstrate a low but measurable permeability to HCO₃⁻. Therefore, under conditions where intracellular Cl⁻ is at or near electrochemical equilibrium then it is possible that CFTR could act as an exit pathway for HCO₃⁻. With this in mind our computer modeling studies indicate that parallel operation of CFTR channels and Cl⁻/HCO₃⁻ exchangers cannot support the secretion of a pancreatic juice containing near isotonic NaHCO₃, as occurs in most other species [13]. Secretory studies on isolated guinea-pig ducts have also virtual absence of extracellular Cl⁻ which would not be predicted for the CFTR – anion exchanger model [14, 15]. The implication of these findings is that species such as cat, dog, pig, guinea-pig and human, all of which secrete a pancreatic juice with a high HCO₃⁻ content (about 150 mM), employ a different secretory mechanism to that originally suggested for the rat, but which is still dependent on CFTR (see the chapter by Sohma et al. which discusses this in more detail [16]).

Extracellular HCO₃⁻ Blocks Cl⁻ Efflux through CFTR

During recent anion permeability studies from native guinea pig PDCs, we observed an
unexpected and novel effect of extracellular 
HCO\textsubscript{3}^- on cAMP-activated CFTR Cl\textsuperscript{-} currents [17]. Figure 1 shows that when 140 mM extracellular Cl\textsuperscript{-} is replaced by HCO\textsubscript{3}^- this resulted in a marked inhibition of CFTR currents. While the reduction in outward current (anion influx) was expected because of the decrease in extracellular Cl\textsuperscript{-} concentration, the marked block of inward current (anion efflux) was not predicted as pipette Cl\textsuperscript{-} concentration was unchanged. The reduced inward current indicates that external HCO\textsubscript{3}^- is causing 'trans' inhibition of Cl\textsuperscript{-} efflux. This effect of extracellular HCO\textsubscript{3}^- was rapid, fully reversible (Figure 2a) and dose-dependent over a physiological range of extracellular HCO\textsubscript{3}^- concentrations (Figure 2b). The data in Figure 2b suggest that a single binding site is involved in the HCO\textsubscript{3}^- induced inhibition of inward current flow. Since inhibition was only weakly voltage-dependent (Figures 1 and 2a), this site is unlikely to experience the voltage drop across the channel. We next investigated which component of the HCO\textsubscript{3}^- containing solutions, pH, HCO\textsubscript{3}^- or pCO\textsubscript{2}, was responsible for the observed current inhibition. By varying intra and extracellular pH over a wide range (6.2-8.0), and changing pCO\textsubscript{2} fourfold (3-12 kPa) while maintaining a concentration of HCO\textsubscript{3}^- that caused maximal inhibition, we were able to conclude that it is the HCO\textsubscript{3}^- ion itself that inhibits CFTR [17]. Although our experiments have not identified how HCO\textsubscript{3}^- is able to block CFTR we think that an external anion-binding site is involved. We speculate that a positively charged site (arginine, lysine or possibly histidine) in the extracellular loops (EL) of CFTR could be
involved (Figure 3). For example in EL1 of human CFTR residues R104 and R117 are conserved amongst all species, and R117H is a known disease causing mutation. Our current research is aimed at testing this hypothesis. It should also be noted that HCO$_3^-$ is not unique in being able to inhibit Cl$^-$ movement through CFTR, since both extracellular $\Gamma$ and ClO$_4^-$ also cause a significant reduction in inward current, but with less affinity than HCO$_3^-$, and in the case of iodide, irreversibly [17].

**Physiological Implications of HCO$_3^-$ Inhibition of CFTR**

At first sight an inhibitory effect of extracellular HCO$_3^-$ on CFTR appears paradoxical in that it would inhibit HCO$_3^-$ secretion. At the maximum concentration of HCO$_3^-$ found in guinea-pig pancreatic juice (about 150 mM) the CFTR conductance would be more than 70% blocked (Figure 2). However, it is notable that in guinea pig ducts basal HCO$_3^-$ secretion is Cl$^-$ dependent and blocked by 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), suggesting that it occurs via Cl$^-$/HCO$_3^-$ exchange [13, 14]. In contrast, cAMP-stimulated HCO$_3^-$ secretion is unaffected by removal of extracellular Cl$^-$ and must therefore involve some other pathway [13, 14]. That pathway is likely to be CFTR. Inhibiting the CFTR conductance via a negative feedback mechanism from ‘signals’ in the lumen of the pancreatic ducts may be advantageous in that it would limit apical membrane depolarisation and maintain the electrical driving force for HCO$_3^-$ secretion via the uninhibited fraction of CFTR. Since many other organ systems (liver, gastro-intestinal tract and lungs) also secrete HCO$_3^-$, this suggests that HCO$_3^-$ concentration at the luminal surface of epithelial cells plays a general role in the regulation of CFTR, as well as providing an appropriate physiological environment for these tissues to operate normally.

**Key words** Chloride Channels; Cystic Fibrosis; Ion Transport; Pancreas; Sodium Bicarbonate

**Abbreviations** CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; EGTA: ethyleneglycolbiss-(beta-aminoethyl ether)-N,N'-tetraacetic acid; EL: extracellular loops; HEPES: N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid; PDC: pancreatic duct cell

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**References**


