JOP. J. Pancreas (Online) 2001; 2(4 Suppl):207-211.

Functional Interactions of HCO_3^- 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 HCO_3^- 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 HCO_3^- transport by so many CF-affected organ systems, it is perhaps surprising that relatively little is known about the interactions of HCO_3^- ions with CFTR. We have used patch clamp recordings from native pancreatic duct cells to study HCO_3^- permeation and interaction with CFTR. Ion selectivity studies shows that CFTR is between 3-5 times more selective for Cl^- over HCO_3^-. In addition, extracellular HCO_3^- has a novel inhibitory effect on cAMP-stimulated CFTR currents carried by Cl^-. The block by HCO_3^- was rapid, relatively independent of voltage and occurred over the physiological range of HCO_3^- concentrations. These data show that luminal HCO_3^- 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 HCO_3^- 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 HCO_3^- [1], glutathione [2] and larger organic anions [3]. In the case of HCO_3^- 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 HCO_3^- secretion the exact role it plays is still uncertain.

Our studies have focused on the role of CFTR in the production of an HCO_3^- rich alkaline secretion by the exocrine pancreas [1]. We

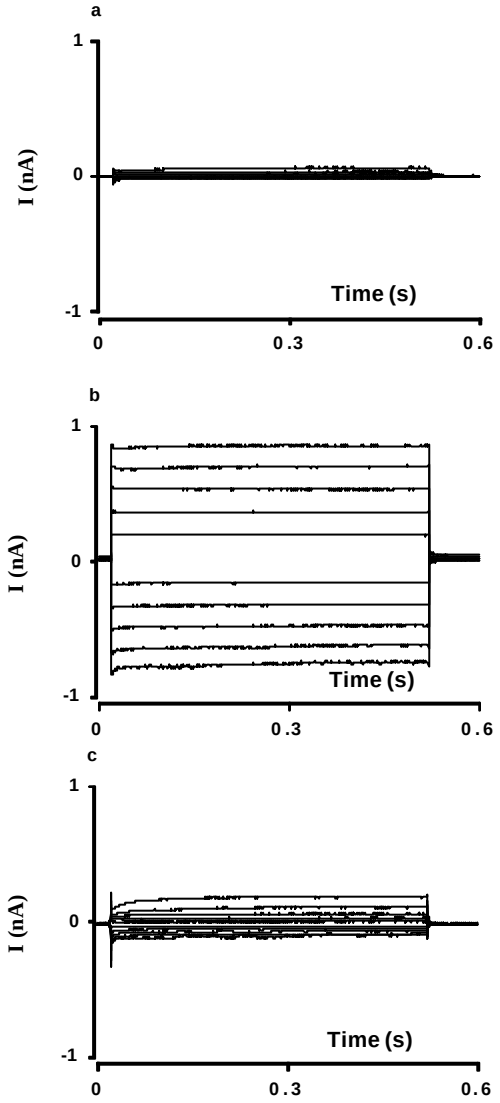

Figure 1. Inhibition of cAMP-activated currents by bath HCO_3^-.
Whole cell currents were recorded at room temperature under control conditions (a) or after exposure to stimulants (5 μM forskolin and 100 μM dibutyryl cAMP) that activate PKA (b and c). Whole cell currents were obtained by holding the membrane potential (V_m) at 0 mV and clamping V_m to ±100 mV in 20 mV steps. The pipette solution contained (mM): 110 CsCl, 2 $MgCl_2$, 5 ethyleneglycol-bis-(beta-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1 Na_2ATP, pH 7.2 with CsOH. The bath solution contained (mM): 145 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 5 Glucose, pH 7.4 or in (c), 140mM NaCl was replaced with $NaHCO_3$ and $CaCl_2$ was omitted from the solution (pH about 8.0). For further details on cell preparation and electrophysiology see [17].

proposed back in 1988 that HCO_3^- exits across the apical membrane of pancreatic duct cells (PDCs) by parallel operation of CFTR Cl^- channels and Cl^-/HCO_3^- 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 HCO_3^- 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 HCO_3^-. 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 HCO_3^-. With this in mind our computer modeling studies indicate that parallel operation of CFTR channels and Cl^-/HCO_3^- exchangers cannot support the secretion of a pancreatic juice containing near isotonic $NaHCO_3$, 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 HCO_3^- 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 HCO_3^- Blocks Cl^- Efflux through CFTR

During recent anion permeability studies from native guinea pig PDCs, we observed an

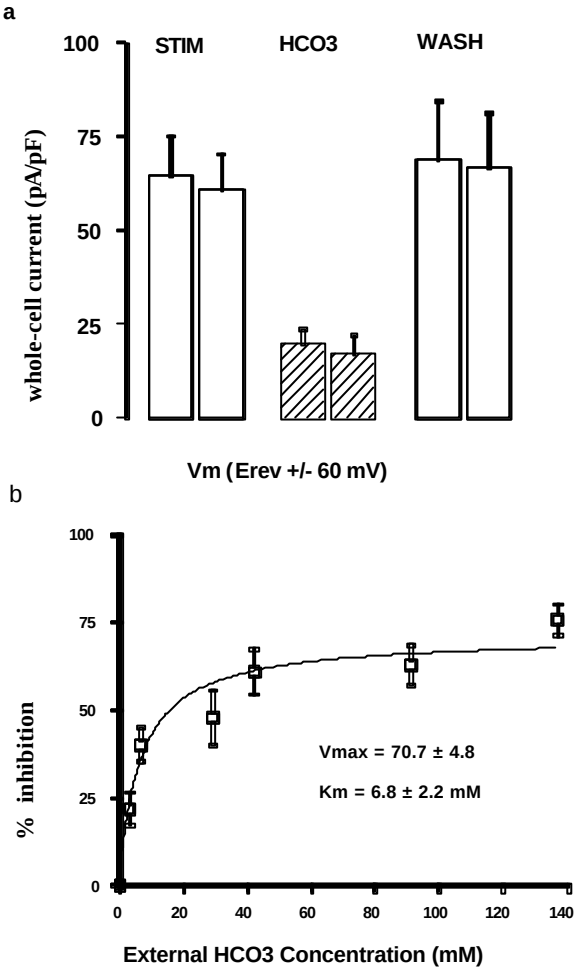

Figure 2. Reversible and concentration-dependent block of CFTR by extracellular HCO_3^-.

(a) Summary of the effect of 140 mM external HCO_3^- on the size of cAMP-activated CFTR Cl^- currents. Same conditions as Figure 1. Current density was calculated by dividing the total current by cell capacitance. Data measured at the reversal potential (E_{rev}) ±60 mV and was obtained from current/voltage plots of the data in Figure 1.

(b) Effect of different extracellular HCO_3^- concentrations on inward current inhibition. Data measured at E_{rev} –60 mV and fitted to a Michaelis-Menten equation with the parameters indicated on the figure (diagram adapted from O'Reilly CM *et al*., with permission [17]).

unexpected and novel effect of extracellular HCO_3^- on cAMP-activated CFTR Cl^- currents [17]. Figure 1 shows that when 140 mM extracellular Cl^- is replaced by HCO_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^- concentration, the marked block of inward current (anion efflux) was not predicted as pipette Cl^- concentration was unchanged. The reduced inward current indicates that external HCO_3^- is causing 'trans' inhibition of Cl^- efflux.

This effect of extracellular HCO_3^- was rapid, fully reversible (Figure 2a) and dose-dependent over a physiological range of extracellular HCO_3^- concentrations (Figure 2b).

The data in Figure 2b suggest that a single binding site is involved in the HCO_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_3^- containing solutions, pH, HCO_3^- or pCO_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_2 fourfold (3-12 kPa) while maintaining a concentration of HCO_3^- that caused maximal inhibition, we were able to conclude that it is the HCO_3^- ion itself that inhibits CFTR [17].

Although our experiments have not identified how HCO_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

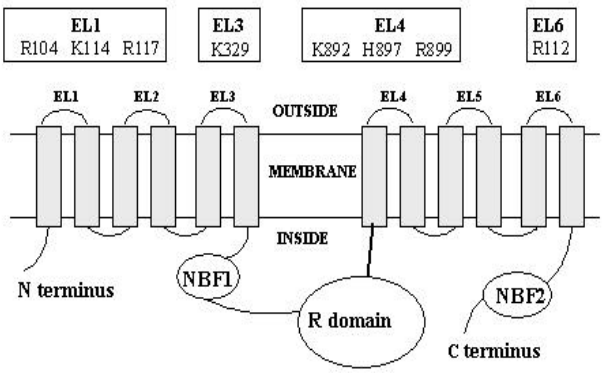

Figure 3. Positively charged residues in the extracellular loops (EL) of human CFTR.

Abbreviations used. H: Histidine, K: Lysine and R: Arginine.

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 I^- 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: ethyleneglycol-bis-(beta-aminoethyl ether)-N,N'-tetraacetic acid; EL: extracellular loops; HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; PDC: pancreatic duct cell

Acknowledgements Funded by grants from the Cystic Fibrosis Trust (UK) and the Wellcome Trust.

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