J Korean Med Sci 2000; 15(Suppl): S12-5
ISSN 1011-8934



# Anion Interactions with CFTR and Consequences for $HCO_3^-$ Transport in Secretory Epithelia

**Mike Gray, Catherine O'Reilly*, John Winpenny†, Barry Argent**

Department of Physiological Sciences, University Medical School, Newcastle upon Tyne, U.K.; *Biomedical Imaging Group, Department of Physiology, University of Massachusetts Medical Centre, Worcester, U.S.A.; †School of Health Sciences, Pasteur Building, University of Sunderland, Sunderland, U.K.

We have been studying CFTR channels in guinea pig pancreatic duct cells and rather surprisingly found that luminal $HCO_3^-$ had a pronounced inhibitory effect on cAMP-activated CFTR chloride currents. The block produced by $HCO_3^-$ was rapid, voltage-independent and occurred over a physiological range of extracellular $HCO_3^-$ concentrations. $I^-$ and $ClO_4^-$ were also found to inhibit CFTR currents, but both were less effective than $HCO_3^-$. Although we have not identified how $HCO_3^-$ is able to block CFTR our data suggests that an external anion-binding site on the channel itself is involved. Overall, our results show that luminal $HCO_3^-$ acts as a potent inhibitor of CFTR channels (and by inference CFTR-mediated secretion), under normal physiological conditions. These data have implications not only for current models of pancreatic duct cell $HCO_3^-$ transport, but also for other bicarbonate-secreting tissues, such as the liver, GI tract and lungs.

## Introduction

It is well established that 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 the submucosal glands (5); the GI 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. Given the importance of CFTR in $HCO_3^-$ transport by so many organ systems, it is perhaps surprising that relatively little is known about the interactions of $HCO_3^-$ ions with CFTR.

**Key Words:** *CFTR; Bicarbonate; Epithelia; Patch Clamp*

**Address for correspondence:** Dr. M A Gray
University Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K., Tel: +44.191-222-7592, Fax: +44.191-222-6706, E-mail: m.a.gray@ncl.ac.uk

## Role of CFTR in pancreatic ductal $HCO_3^-$ transport

We proposed back in 1988 that $HCO_3^-$ secretion across the apical membrane of pancreatic duct cells (PDC's) occurs by parallel operation of CFTR $Cl^-$ channels and $Cl^-/HCO_3^-$ exchangers (10). This CFTR–anion exchanger model has been largely derived from studies on rat PDC's (11) which secrete a relatively low concentration of $HCO_3^-$ (~70 mM). 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 (12). Secretory studies on isolated guinea-pig ducts have also shown that $HCO_3^-$ secretion can occur in the virtual absence of extracellular $Cl^-$ which would not be predicted for the CFTR–anion exchanger model (13, 14). 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 (~150 mM), employ a different secretory mechanism to that originally suggested for the rat.

## Activation of CFTR currents in pancreatic duct cells

In an attempt to resolve this issue we have studied CFTR channels in guinea pig PDC's (17). Exposing these cells to a maximal dose of either secretin or to a mixture of forskolin/dibutyryl cAMP, stimulates $Cl^-$ selective currents in ~75% of cells tested. However, in contrast to rat and mouse PDC's (15, 16), two kinetically distinct currents are activated (Fig. 1). In over 70% of responding cells the currents are time- and voltage-independent and thus have classical CFTR properties. Whole-cell currents increase from basal values of 5-10 pA/pF to ~75 pA/pF at 60 mV. In the remaining cells distinct outwardly rectifying, time- and voltage-dependent currents are activated ($Cl_{tv\ dep}$). These display marked tail-currents and have very large current densities (~1,000 pA/pF).

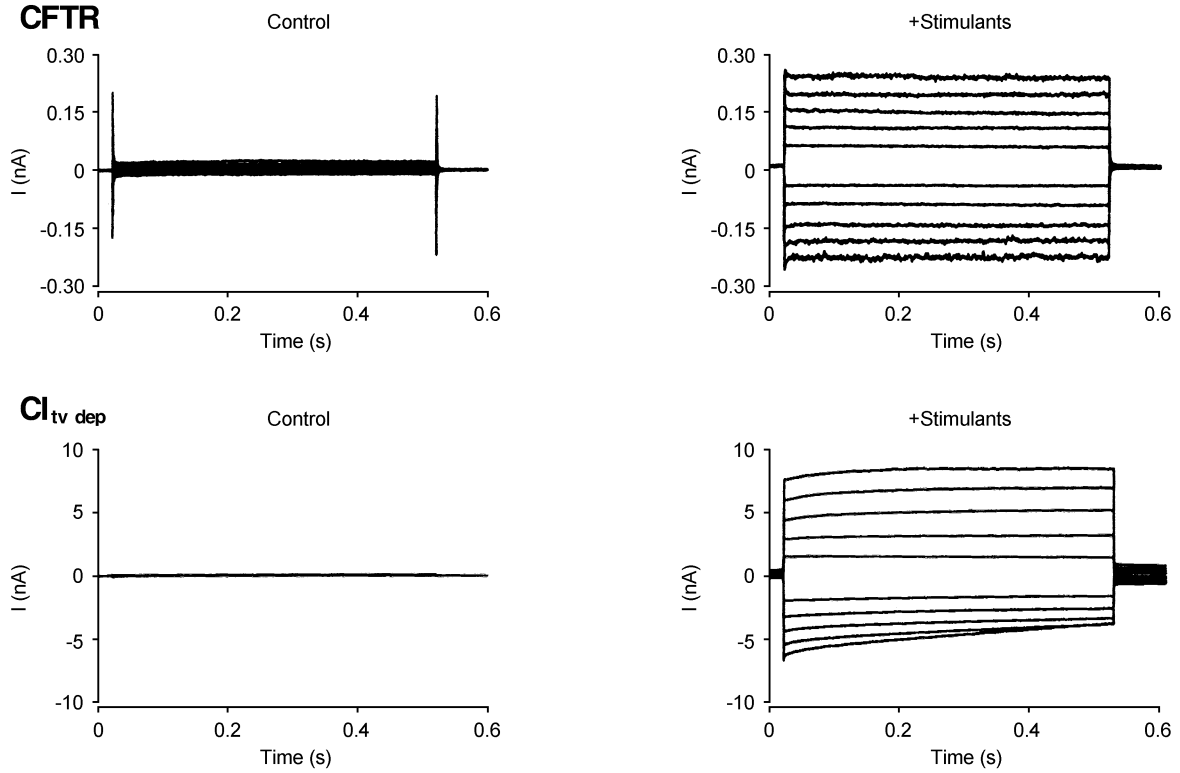


Fig. 1. Two types of whole-cell $Cl^-$ currents activated by cAMP from guinea-pig pancreatic duct cells. Whole cell currents were recorded under control conditions or after exposure to stimulants (5 $\mu$M forskolin and 100 $\mu$M dibutyryl cAMP). Note the different vertical scales for CFTR and $Cl_{tv\text{-}dep}$ currents and the time- and voltage-dependent kinetics of the $Cl_{tv\text{-}dep}$. Whole cell currents were obtained by holding $V_m$ at 0 mV and clamping to $\pm$100 mV in 20 mV steps. The pipette solution contained (mM): 110 CsCl, 2 $MgCl_2$, 5 EGTA, 10 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.

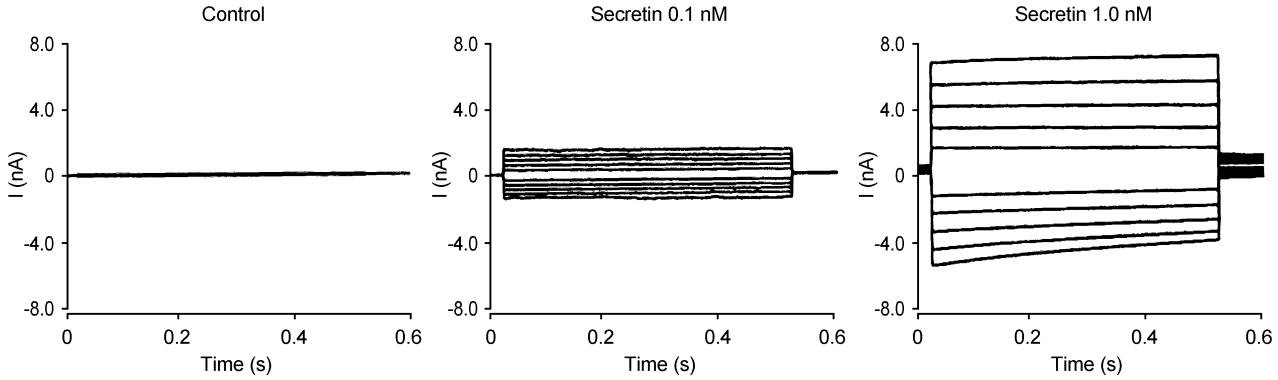


Fig. 2. Conversion of CFTR currents into $Cl_{tv\text{-}dep}$ during exposure to secretin. Same conditions as in Fig. 1, except a single cell was exposed to increasing concentrations of secretin.

Although the two currents look very different, we believe that CFTR $Cl^-$ channels underlie both conductances. Both currents have identical anion permeability sequences ($Br^->I^-=Cl^->HCO_3^->ClO_4^->$Aspartate), are not blocked by DIDS (0.5 mM), but are inhibited (~60 %) by glibenclamide (0.1 mM). In addition, some cells exhibit typical CFTR currents at low doses of stimulants, but Cltv-dep currents at higher doses (Fig. 2). We believe that a minority of cells express CFTR at very high levels and it is this high density of channels in the apical membrane that causes the time- and voltage-dependent kinetics. Note that the kinetics of the Cltv-dep currents are most marked on the inward currents (Fig. 1&2), and this is consistent with $Cl^-$ ion depletion at the internal face of the CFTR channels caused by the large inward current flow (see 17 for further discussion). Other than

the occasional presence of these large CFTR currents, we have not found any major differences between guinea pig PDC's compared to rat or mouse PDC's. Whether the large CFTR $Cl^-$ currents can explain the species-dependent differences in bicarbonate transport is unclear at the moment.

### Effect of $HCO_3^-$ ions on CFTR currents

During the anion-substitution experiments, we observed a novel effect of $HCO_3^-$ on the cAMP-activated currents (17). Fig. 3 shows that extracellular $HCO_3^-$ (150 mM $Cl^-$ replaced by $HCO_3^-$) causes a marked inhibition of the CFTR currents (identical results were obtained for $Cl_{rv\text{-}dep}$). This effect of $HCO_3^-$ is rapid, dose-dependent over the physiological range of extracellular $HCO_3^-$ concentrations (Fig. 3D), and is fully reversible. Note that extracellular $HCO_3^-$ inhibits both the inward current (anion efflux) and outward current (anion influx) through CFTR (Fig. 3A, B&C). The reduced inward current indicates that external $HCO_3^-$ is causing 'trans' inhibition of $Cl^-$ efflux. Our experiments suggest that a single binding site is involved in $HCO_3^-$ inhibition (Fig. 1D). Since inhibition was only weakly voltage-dependent (Fig. 3B&C), this site is unlikely to experience the voltage drop across the channel.

We 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, and changing $pCO_2$, at a constant $HCO_3^-$, we conclude that it is the $HCO_3^-$ ion itself that inhibits CFTR. Although our experiments have not identified how $HCO_3^-$ is able to block CFTR we suggest that an external anion-binding site is involved. In this regard our data show that $HCO_3^-$ is not unique in being able to inhibit $Cl^-$ movement through

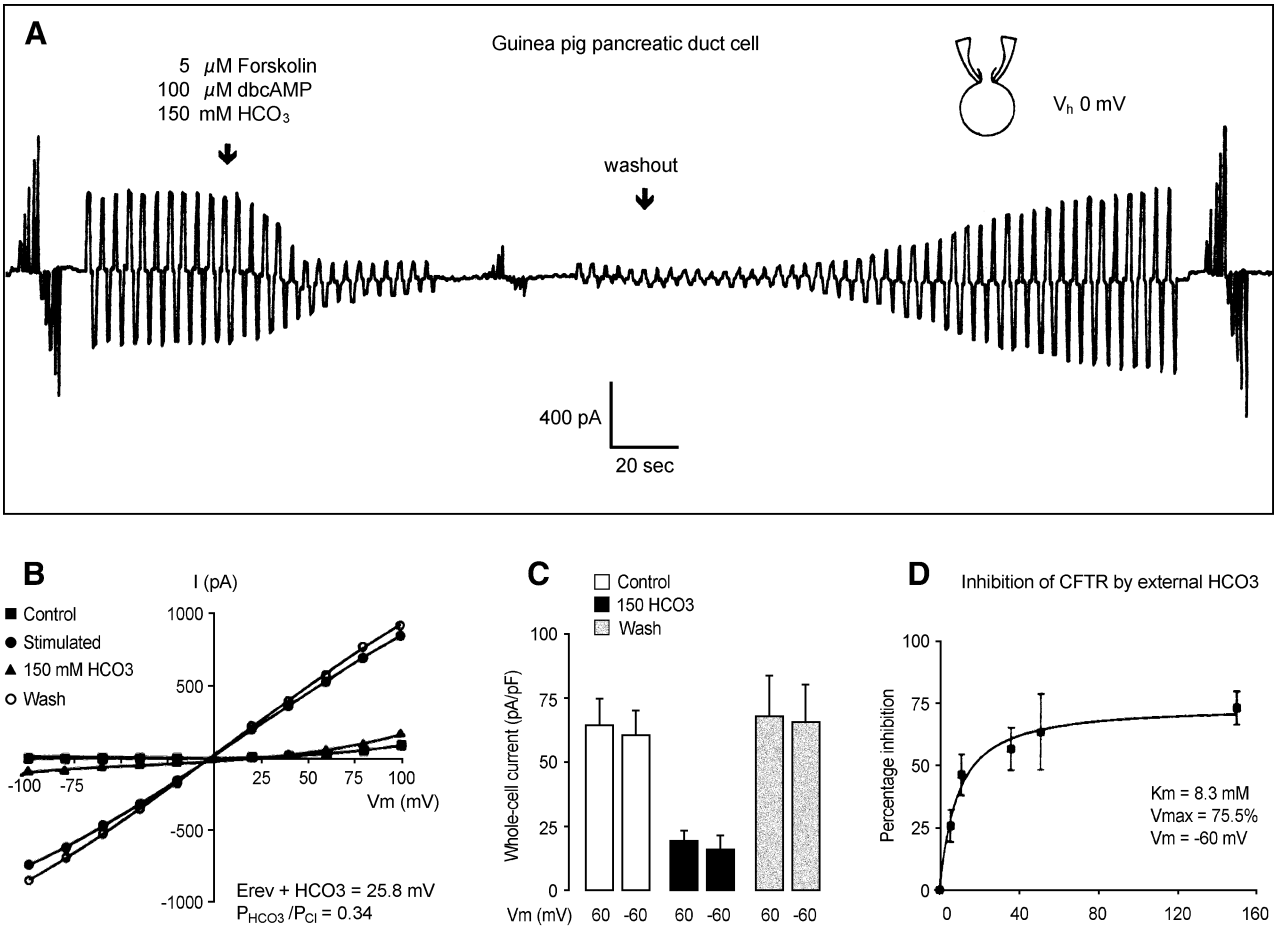


Fig. 3. Inhibition of cAMP-activated currents by bath $HCO_3^-$. (A) Continuous whole cell recording showing the effect of bath application of 150 mM $HCO_3^-$ ($pH_o$ ~8.0: 5.5 mM $Cl^-$ remaining) on CFTR currents previously activated by cAMP stimulants. Currents were obtained by holding $V_m$ at 0 mV and clamping to ±60 mV. (B) I/V plots for the data in A using the to ±100 mV protocol. Stimulated currents were inhibited by ~80%. (C) Summary of the effect of 150 mM external $HCO_3^-$ on the size of CFTR currents. (D) Effect of a range of $HCO_3^-$ concentrations on current inhibition. Data were fitted to a Michaelis-Menten equation with the parameters indicated on the figure.

CFTR, since both extracellular $I^-$ and $ClO_4^-$ ions also cause a significant reduction in inward current, but with less affinity than $HCO_3^-$.

## 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 (~150 mM) the CFTR conductance would be 80% blocked (Fig. 3). However, it is notable that in guinea-pig ducts basal $HCO_3^-$ secretion is $Cl^-$ dependent and blocked by 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 the lumen may be advantageous in that it would limit apical membrane depolarization and maintain the electrical driving force for $HCO_3^-$ secretion via the uninhibited fraction of CFTR. Since many other organ systems (liver, GI tract and lungs) also secrete $HCO_3^-$ to some extent, this leaves open the possibility that $HCO_3^-$ concentration at the luminal surface of epithelial cells plays a general role in the regulation of CFTR.

## References


1. Argent BE, Gray MA. *Regulation and formation of fluid and electrolyte secretions by pancreatic ductal epithelium. In: Sirica AE, Longnecker DS, eds. Biliary and pancreatic ductal epithelia. Pathobiology and pathophysiology. New York: Marcel Dekker, 1997; 349-77.*
2. Linsdell P, Hanrahan JW. *Glutathione permeability of CFTR. Am J Physiol 1998; 275: C323-6.*
3. Linsdell P, Hanrahan JW. *ATP-dependent asymmetry of anion permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. J Gen Physiol 1998; 111: 601-14.*
4. Smith JJ, Welsh MJ. *cAMP stimulates bicarbonate secretion across normal but not cystic fibrosis airway epithelia. J Clin Invest 1992; 89: 1148-53.*
5. Ballard ST, Trout L, Bebok Z, Sorscher EJ, Crews A. *CFTR involvement in chloride, bicarbonate, and liquid secretion by airway submucosal glands. Am J Physiol 1999; 277: L694-9.*
6. Hogan, DL, Crombie DL, Isenberg JI, Svendsen P, Schaffalitzky de Muckadell OB, Ainsworth MA. *CFTR mediates cAMP- and $Ca^{2+}$-activated duodenal epithelial $HCO_3^-$ secretion. Am J Physiol 1997; 272: G872-8.*
7. Fitz JG, Cohn JA. *Biology and pathophysiology of CFTR and other $Cl^-$ channels in biliary epithelial cells. In: Sirica AE, Longnecker DS, eds. Biliary and pancreatic ductal epithelia. Pathobiology and pathophysiology. New York: Marcel Dekker, 1997; 107-25.*
8. Curtis CM, Martin LC, Higgins CF, Colledge WH, Hickman ME, Evans MJ, MacVinish LJ, Cuthbert AW. *Restoration by intratracheal gene transfer of bicarbonate secretion in cystic fibrosis mouse gallbladder. Am J Physiol 1998; 274: G1053-60.*
9. Durie PR, Forstner GG. *Pathophysiology of the exocrine pancreas in cystic fibrosis. J R Soc Med 1989; 82(Suppl 16): 2-10.*
10. Gray MA, Greenwell JR, Argent BE. *Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. J Membr Biol 1988; 105: 131-42.*
11. Argent BE, Case RM. *Pancreatic ducts. Cellular mechanism and control of bicarbonate secretion. In: Johnson LR, ed. Physiology of the gastrointestinal tract. 3rd ed. New York: Raven, 1994; 2: 1473-97.*
12. Sohma Y, Gray MA, Imai Y, Argent BE. *A mathematical model of the pancreatic ductal epithelium. J Membr Biol 1996; 154: 53-67.*
13. Ishiguro H, Steward MC, Wilson RW, Case RM. *Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495: 179-91.*
14. Ishiguro H, Naruse S, Steward MC, Kitagawa M, Ko SBH, Hayakawa T, Case RM. *Fluid secretion in interlobular ducts isolated from guinea-pig pancreas. J Physiol 1998; 511: 407-22.*
15. Gray MA, Plant S, Argent BE. *cAMP-regulated whole cell chloride currents in pancreatic duct cells. Am J Physiol 1993; 264: C591-602.*
16. Gray MA, Winpenny JP, Porteous DJ, Dorin JR, Argent BE. *CFTR and calcium-activated chloride currents in pancreatic ducts of a transgenic CF mouse. Am J Physiol 1994; 266: C213-21.*
17. O'Reilly C, Winpenny J, Argent BE, Gray MA. *Cystic fibrosis transmembrane conductance regulator currents in guinea-pig pancreatic duct cells and their inhibition by bicarbonate ions. Gastroenterolgy 2000. (in press)*