*JOP. J. Pancreas (Online) 2001; 2(4 Suppl):192-197.*

# Pancreatic Ductal Bicarbonate Secretion: Past, Present and Future

**Hiroshi Ishiguro[1], Satoru Naruse[1], José I San Román[2,3], Maynard Case[2], and Martin C Steward[2]**

[1]Departments of Internal Medicine II and Human Nutrition, Nagoya University School of Medicine. Nagoya, Japan, [2]School of Biological Sciences, University of Manchester. United Kingdom. [3]Departamento de Fisiología y Farmacología, Universidad de Salamanca. Salamanca, Spain

## Summary

The pancreatic duct epithelium in the guinea-pig and many other species secretes $HCO_3^-$ at concentrations approaching 150 mM. This cannot be explained by conventional models based upon $HCO_3^-$ secretion via an anion exchanger at the luminal membrane because: 1) under these conditions, the $Cl^-$ and $HCO_3^-$ concentration gradients would favour $HCO_3^-$ reabsorption rather than secretion, and 2) the luminal anion exchanger appears to be inhibited by luminal $HCO_3^-$ concentrations of 125 mM or more. There may, however, be a sufficiently large electrochemical gradient to drive $HCO_3^-$ secretion across the luminal membrane *via* an anion conductance. In contrast to earlier studies on rat ducts, the membrane potential $E_m$ in guinea-pig duct cells does not depolarise appreciably upon stimulation with secretagogues but remains constant at about –60 mV. Consequently, even with 125 mM or more $HCO_3^-$ in the lumen and an estimated 20 mM in the cytoplasm, the electrochemical gradient for $HCO_3^-$ will still favour secretion to the lumen. Under the same conditions, the intracellular $Cl^-$ concentration drops to very low levels (approximately 7 mM) presumably because, although $Cl^-$ may leave freely through the cystic fibrosis transmembrane conductance regulator (CFTR) channels in the luminal membrane, there is no major pathway for $Cl^-$ uptake across the basolateral membrane. Consequently a $HCO_3^-$-rich secretion may arise as a result of the lack of competition from intracellular $Cl^-$ for efflux via the anion conductances at the luminal membrane. Whether CFTR, or another anion conductance, provides such a pathway for $HCO_3^-$ remains to be seen.

## Introduction

When stimulated with secretin, pancreatic ducts secrete an isotonic, $HCO_3^-$-rich fluid [1]. It has been widely believed that $HCO_3^-$ is generated from $CO_2$ in the epithelial cells by a basolateral $Na^+/H^+$ exchanger working to extrude protons generated from the hydration of $CO_2$ by carbonic anhydrase. $HCO_3^-$ secretion across the luminal membrane is then thought to occur by exchange for $Cl^-$ on an anion exchanger working in parallel with the CFTR $Cl^-$ channel. However, this model is based largely on experimental studies of rat pancreatic ducts [2, 3] which secrete a mixture of $Cl^-$ and $HCO_3^-$, the latter achieving concentrations generally no higher than about 70 mM [4].

In the guinea-pig, and in many other species, the ducts secrete $HCO_3^-$ at much higher concentrations, often approaching 150 mM [5]. It can be argued on thermodynamic grounds that such $HCO_3^-$ concentrations could not be achieved by the transport mechanism described above.

Recent work has suggested a number of modifications to the original model which may help to explain how the pancreatic ducts in species such as the guinea-pig achieve such high secreted $HCO_3^-$ concentrations [6]. Some of these modifications are supported by experimental findings, others are not. Furthermore, a crucial unanswered question concerns the precise role of CFTR in $HCO_3^-$ secretion. Pancreatic insufficiency in cystic fibrosis is generally attributed to a failure of $HCO_3^-$ secretion in the pancreatic ducts, and yet the CFTR channel itself shows a marked selectivity to $Cl^-$ over $HCO_3^-$.

At the basolateral membrane, the situation seems to be relatively clear. While basolateral $HCO_3^-$ uptake may be mediated in part by a basolateral $Na^+/H^+$ exchanger (NHE), studies of intracellular pH ($pH_i$) in guinea-pig ducts show that there is also a major contribution from a $Na^+$-$HCO_3^-$ cotransporter (NBC) at the basolateral membrane [7]. Thus, in duct cells loaded with the pH-sensitive fluoroprobe 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF), the recovery of $pH_i$ from an acid load in the absence of $HCO_3^-$ is totally abolished by treatment with amiloride, an inhibitor of $Na^+/H^+$ exchange. In the presence of $HCO_3^-$, however, the recovery process is only partially inhibited by amiloride and there is an additional, $Na^+$- and $HCO_3^-$-dependent component that is sensitive to dihydro-4,4'-diisothyocyanatostilbene-2,2'-disulphonic acid ($H_2$DIDS). Our proposal that this is mediated by a $Na^+$-$HCO_3^-$ cotransporter is supported by other functional studies and by immunohistochemical studies confirming that an NBC is expressed in the basolateral membranes of rat and human pancreatic duct cells [8, 9].

Although the presence of an $H^+$-ATPase has also been demonstrated in both rat and guinea-pig pancreatic duct cells [10, 11], our data suggest that, in guinea-pig ducts, the uptake of $HCO_3^-$ at the basolateral membrane during secretin or forskolin stimulation can be largely attributed to the NBC and NHE, with the former predominating 3:1 over the latter during maximal stimulation. Since there is evidence that NBC is also expressed in rat pancreatic ducts [10], it seems doubtful that the involvement of the NBC *per se* accounts for the secretion of the much higher $HCO_3^-$ concentrations observed in the guinea-pig ducts.

At the luminal membrane, the situation is far less clear. The efflux of $HCO_3^-$ from the cells to the lumen could, in principle, be mediated a) by neutral exchange for $Cl^-$, b) by electrodiffusion through an anion conductance or c) by an as yet unidentified transporter. However, our previous work on guinea-pig ducts has indicated that, although spontaneous secretion certainly involves anion exchange at the luminal membrane, and is dependent on luminal $Cl^-$ and inhibited by $H_2$DIDS, secretin-evoked secretion can occur in the nominal absence of luminal $Cl^-$ (less than 8 mM) and is not blocked by $H_2$DIDS [12, 13].

Our present work is therefore focused on measuring the concentration and electrical potential gradients for $Cl^-$ and $HCO_3^-$ across the luminal membrane, the aim being to establish a) whether the gradients that exist under physiological conditions could drive electrodiffusive secretion of $HCO_3^-$ through a luminal membrane anion conductance, and b) why the guinea-pig ducts secrete $HCO_3^-$ in preference to $Cl^-$. Put another way, why do guinea-pig ducts secrete so little $Cl^-$?

## Methods

Isolated interlobular ducts were microperfused using a concentric pipette arrangement consisting of an outer, constricted holding pipette, a perfusion pipette and an inner exchange pipette to obtain rapid exchange of the luminal perfusate. Pressurised nitrogen gas was applied to the

reservoirs supplying the luminal perfusion solutions, and the waste line was connected to a reservoir located approximately 30 cm above the chamber. The bath was continuously perfused in the same direction as the flow of luminal perfusate, so that the luminal solution leaving the open end of the duct was swept away by the flow of the bath solution.

Intracellular $Cl^-$ concentration was estimated by microfluorometry in ducts loaded with the $Cl^-$-sensitive fluoroprobe, 6-methoxy-N-ethylquinolinium chloride (MEQ). Dihydro-MEQ (diH-MEQ), the membrane-permeable form of MEQ, was synthesized from MEQ and the duct segments incubated for 30 min at room temperature with 100 μM diH-MEQ. Microfluorometry was performed with excitation at 340 nm and the fluorescence was measured at 430 nm. Calibration was performed *in situ* by application of a combination of nigericin (5 mM) and tributyltin chloride (10 mM). The Stern-Volmer constant was 11 $M^{-1}$.

To measure membrane potential, the basolateral membrane was impaled with glass microelectrodes filled with 0.5 M KCl and connected to an electrometer (World Precision Instruments Inc., Sarasota, FL, USA). The intracellular potential ($E_m$) was measured with reference to the grounded bath. Fluid secretion was measured in isolated interlobular ducts from guinea-pig and rat pancreas by video microscopy. Bright-field images were captured on an inverted microscope at regular intervals and the luminal volume of the ducts estimated from image area occupied by the luminal space. Fluid secretory rate was estimated from the rate of increase in luminal volume.

## Results and Discussion

In these studies we have used a microperfusion technique to control independently the composition of the luminal and basolateral solutions bathing interlobular duct segments isolated from the guinea-pig pancreas. This has been combined with microfluorometry to measure $pH_i$ and intracellular $Cl^-$ concentration ($[Cl^-]_i$) with the fluoroprobes BCECF and MEQ respectively. Membrane potentials ($E_m$) have been determined using conventional glass microelectrodes. Measurements of all three variables have been made in both unstimulated and forskolin- (or dibutyryl-cAMP-) stimulated ducts, with both high $Cl^-$ and high $HCO_3^-$ solutions in the lumen.

Measurements of $pH_i$ in microperfused ducts have confirmed the distribution of transporters predicted by our earlier studies [14]. Thus we observe that NBC and NHE activities are confined to the basolateral membrane while anion exchangers (AEs) can be detected in both luminal and basolateral membranes, and we have found no evidence of any other acid/base transporters in either membrane. $Cl^-$ substitution experiments suggest that, in unstimulated cells, the activity of the basolateral AE exceeds that of the luminal AE, suggesting that this might provide a significant, and perhaps the only, route for $Cl^-$ uptake across the basolateral membrane. While secretin stimulation appears to activate the luminal AE, raising the luminal concentration of $HCO_3^-$ has the opposite effect, inhibiting the AE and thereby helping to prevent the reabsorption of secreted $HCO_3^-$.

Using the $Cl^-$-sensitive fluoroprobe MEQ, the value of $[Cl^-]_i$ in unstimulated duct cells bathed on both sides by high $Cl^-$/low $HCO_3^-$ solutions (25 mM $HCO_3^-$, 124 mM $Cl^-$) was approximately 30 mM. Under similar conditions the membrane potential, measured with conventional glass microelectrodes, was approximately -60 mV. The value of $[Cl^-]_i$ therefore exceeds the value predicted for electrochemical equilibrium (*circa* 12 mM) and this indicates that $Cl^-$ is actively accumulated in the cells. This is most probably the result of exchange with intracellular $HCO_3^-$ since the value of $[Cl^-]_i$ falls in the absence of $HCO_3^-$ or upon addition of $H_2$DIDS either to the luminal solution or, even

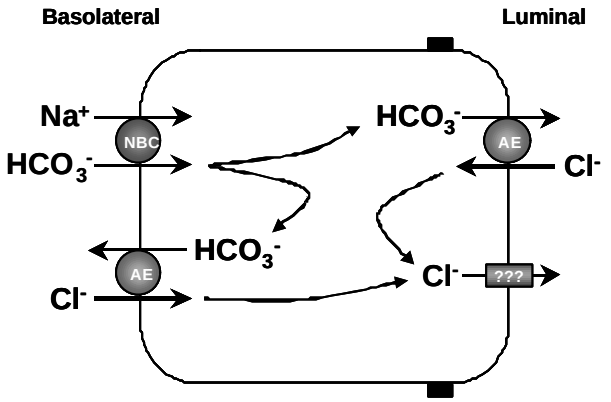


**Figure 1.** Predicted anion fluxes in an unstimulated guinea-pig pancreatic duct cell bathed on both sides by high $Cl^-$-low $HCO_3^-$ solutions.

more so, to the bath solution. These data support our previous conclusion [14] that anion exchangers are present in both the luminal and basolateral membranes and they also indicate that the basolateral exchanger is more active than the luminal exchanger in the unstimulated cells. In unstimulated conditions, therefore, we propose that the steady-state fluxes of $Cl^-$ and $HCO_3^-$ are as shown in Figure 1. $HCO_3^-$ uptake is mediated by the basolateral NBC (and also indirectly by a basolateral NHE) and $HCO_3^-$ efflux occurs at both the luminal and basolateral membranes in exchange for the uptake of $Cl^-$. $Cl^-$ in turn leaves the cells *via* an anion conductance, probably CFTR, in the luminal membrane. The net result is a small, spontaneous secretion containing both $Cl^-$ and $HCO_3^-$.

Upon stimulation with secretin or forskolin, the steady-state value of $[Cl^-]_i$ increased by about 5 mM indicating that the stimulation of $Cl^-$ uptake more than compensates for the increased efflux of $Cl^-$ *via* the luminal membrane CFTR $Cl^-$ conductance. From the rate of change of $[Cl^-]_i$ upon substitution of luminal or basolateral $Cl^-$, we deduce that the increase in $Cl^-$ uptake with stimulation occurs mainly at the luminal anion exchanger. Under similar conditions, the membrane potential showed a transient hyperpolarisation upon stimulation with dibutyryl cAMP and this was followed by a small but sustained depolarisation of about 10 mV. These changes are probably the net result of the depolarising effect of increased $Cl^-$ efflux via the luminal CFTR conductance combined with the hyperpolarizing effect of increased electrogenic NBC activity at the basolateral membrane. Our data suggest that there is little or no increase in the activity of the basolateral anion exchanger, and that it may even be inhibited by stimulation with cAMP-raising secretagogues.

The result of these changes is illustrated in Figure 2. Stimulation appears to cause a switch from a small mixed secretion, driven in part by the basolateral uptake of $Cl^-$, to a copious $HCO_3^-$-rich secretion, driven largely by the basolateral NBC. At the luminal membrane, anion exchange is stimulated by cAMP, and $HCO_3^-$ secretion occurs in exchange for luminal $Cl^-$. The supply of $Cl^-$ for this process is derived mainly from the luminal fluid since there is little $Cl^-$ uptake at the basolateral membrane. Under the conditions of these experiments, the supply of $Cl^-$ is unlimited because the lumen is continuously perfused with a high $Cl^-$ solution. However, under physiological conditions, the composition of the luminal fluid is mainly determined by what the duct cells secrete, although there may be a small amount of $Cl^-$ secreted upstream by the acinar cells. Consequently, the

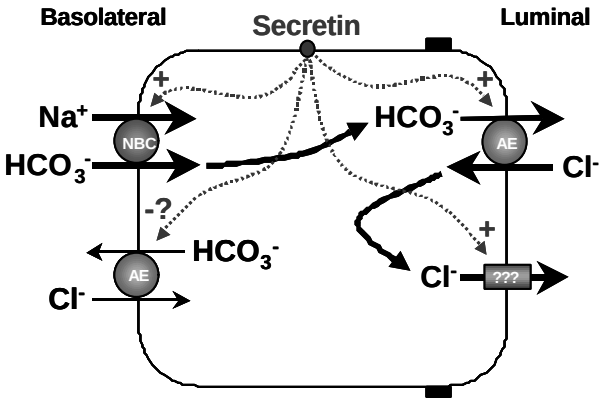


**Figure 2.** Predicted anion fluxes in a guinea-pig pancreatic duct cell stimulated with secretin and bathed on both sides by high $Cl^-$-low $HCO_3^-$ solutions.

secretion of a $HCO_3^-$-rich fluid by the ducts will cause a rise in luminal $HCO_3^-$ concentration, both with time after stimulation, and with distance along the ducts. Above a concentration of about 70 mM, however, the gradients driving the luminal anion exchanger will reverse and the duct cells would be expected to reabsorb $HCO_3^-$ in exchange for $Cl^-$ secretion.

To investigate this situation, experiments were performed on ducts stimulated with forskolin or dibutyryl cAMP and perfused through the lumen with a solution containing 125 mM $HCO_3^-$ and 24 mM $Cl^-$. Under these conditions, $[Cl^-]_i$ was found to decrease to approximately 7 mM, most probably as a result of the reduced uptake of $Cl^-$ by anion exchange at the luminal membrane. From our previous work [14], we believe that this is a consequence of the inhibitory effect of the high luminal $HCO_3^-$ concentration on the luminal membrane anion exchanger. Under similar conditions the membrane potential was found to remain at about -60 mV during stimulation. This means that even with 125 mM $HCO_3^-$ in the lumen and approximately 20 mM $HCO_3^-$ in the cells (since $pH_i$ is approximately 7.2) there is still a net electrochemical gradient favouring $HCO_3^-$ efflux across the luminal membrane. Thus, with a sufficiently large $HCO_3^-$ conductance at the luminal membrane and given the low level of intracellular $Cl^-$, there may be no need to invoke any additional luminal membrane transporter to account for high concentrations of $HCO_3^-$ secreted by the guinea-pig ducts. The situation is summarised in Figure 3. It remains to be seen a) whether such a $HCO_3^-$ conductance exists at the luminal membrane and b) whether it is mediated by CFTR or another anion channel.

Finally, it seems that in the guinea-pig ducts the capacity for $Cl^-$ uptake across the basolateral membrane is limited, with the result that the intracellular concentration of $Cl^-$ falls to very low values during maximal secretion. It is possible that

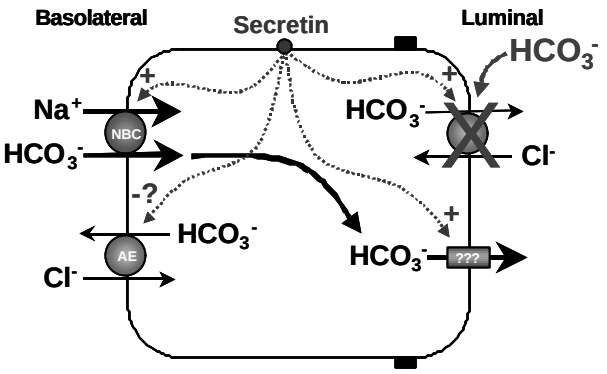


**Figure 3.** Predicted anion fluxes in a guinea-pig pancreatic duct cell stimulated with secretin and bathed on the luminal side by a low $Cl^-$-high $HCO_3^-$ solution.

the only transporter mediating $Cl^-$ uptake across the basolateral membrane is the anion exchanger, and even this may be inhibited during stimulation. It is therefore worth considering whether the difference in the relative $Cl^-$ and $HCO_3^-$ concentrations secreted by guinea-pig and rat ducts may reflect differences at the basolateral membrane rather than at the luminal membrane. In other words, the guinea-pig ducts may be good $HCO_3^-$ secretors because they are poor $Cl^-$ secretors. Some evidence to support this hypothesis comes from measurements of fluid secretion in isolated interlobular ducts from the two species. Fluid secretion in the guinea-pig ducts is totally dependent upon the presence of $HCO_3^-/CO_2$ in the bathing medium. In the rat ducts, however, forskolin stimulation evokes fluid secretion in the absence of $HCO_3^-/CO_2$. Furthermore, this $HCO_3^-$-independent secretion is inhibited by bumetanide, an inhibitor of the $Na^+$-$K^+$-2$Cl^-$ cotransporter (NKCC). It therefore seems plausible that a basolateral NKCC supports $Cl^-$ secretion in the rat ducts, and hence the production of a mixed secretion containing comparable concentrations of $Cl^-$ and $HCO_3^-$ whereas the absence of the NKCC in the guinea-pig ducts ensures that the secretion is $HCO_3^-$-rich.

**Key words** Pancreas; Cystic Fibrosis; Cystic Fibrosis Transmembrane Conductance Regulator; Fluids and Secretions

**Abbreviations** AE: anion exchanger; BCECF: 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; CFTR: cystic fibrosis transmembrane conductance regulator; $[Cl^-]_i$: intracellular $Cl^-$ concentration; diH-MEQ: dihydro-6-methoxy-N-ethylquinolinium chloride; $E_m$: membrane potential; $H_2$DIDS: dihydro-4,4'-diisothyocyanatostilbene-2,2'-disulphonic acid; MEQ: 6-methoxy-N-ethylquinolinium chloride; NBC: $Na^+$-$HCO_3^-$ cotransporter; NHE: $Na^+/H^+$ exchanger; NKCC: $Na^+$-$K^+$-$2Cl^-$ cotransporter; $pH_i$: intracellular pH

**Acknowledgements** Supported by the Wellcome Trust, UK Cystic Fibrosis Trust, the Ministry of Education, Science, and Culture (Japan), the Ministry of Health and Welfare (Japan), and Uehara Memorial Foundation. We thank Dr. Y. Sohma for helpful discussions.

**Correspondence**
Martin C Steward
School of Biological Sciences
University of Manchester
G.38 Stopford Building
Manchester M13 9PT
UnitedKingdom.
Phone: +44-161-275-5455
Fax: +44-161-275-5600
E-mail address: martin.steward@man.ac.uk

**References**

1. Case RM, Argent BE. Pancreatic Duct Secretion: Control and Mechanisms of Transport. In: The Pancreas: Biology, Pathobiology, and Disease. Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA, eds. New York, NY, USA: Raven Press, 1993: 301-50.

2. Gray MA, Greenwell JR, Argent BE. Secretin-regulated chloride channels on the apical plasma membrane of pancreatic duct cells. J Membr Biol 1988; 105:131-42. [89110954]

3. Novak I, Greger R. Properties of the luminal membrane of isolated perfused rat pancreatic ducts: effect of cyclic AMP and blockers of chloride transport. Pflugers Arch 1988; 411:546-53. [88262412]

4. Sewell WA, Young JA. Secretion of electrolytes by the pancreas of the anaesthetized rat. J Physiol 1975; 252:379-96. [76097165]

5. Padfield PJ, Garner A, Case RM. Patterns of pancreatic secretion in the anaesthetised guinea pig following stimulation with secretin, cholecystokinin octapeptide, or bombesin. Pancreas 1989; 4:204-9. [89331499]

6. Sohma Y, Gray MA, Imai Y, Argent BE. $HCO_3^-$ transport in a mathematical model of the pancreatic duct. J Membr Biol 2000; 176:77-100. [20341825]

7. Ishiguro H, Steward MC, Lindsay AR, Case RM. Accumulation of intracellular $HCO_3^-$ by $Na^+$-$HCO_3^-$ cotransport in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495:169-78. [97019905]

8. Marino CR, Jeanes V, Boron WF, Schmitt BM. Expression and distribution of the $Na^+$-$HCO_3^-$ cotransporter in human pancreas. Am J Physiol 1999; 40:G487-94. [99375119]

9. Thevenod F, Roussa E, Schmitt BM, Romero MF. Cloning and immunolocalization of a rat pancreatic $Na^+$ bicarbonate cotransporter. Biochem Biophys Res Commun 1999; 264:291-8. [99458660]

10. Zhao H, Star RA, Muallem S. Membrane localization of $H^+$ and $HCO_3^-$ transporters in the rat pancreatic duct. J Gen Physiol 1994; 104:57-85. [95053869]

11. De Ondarza J, Hootman SR. Confocal microscopic analysis of intracellular pH regulation in isolated guinea pig pancreatic ducts. Am J Physiol 1997; 272:G124-34. [97190926]

12. Ishiguro H, Steward MC, Wilson RW, Case RM. Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495:179-91. [97019906]

13. Ishiguro H, Naruse S, Steward MC, Kitagawa M, Ko SB, Hayakawa T, Case RM. Fluid secretion in interlobular ducts isolated from guinea-pig pancreas. J Physiol 1998; 511:407-22. [98372828]

14. Ishiguro H, Naruse S, Kitagawa M, Suzuki A, Yamamoto A, Hayakawa T, et al. $CO_2$ permeability and bicarbonate transport in microperfused interlobular ducts isolated from guinea-pig pancreas. J Physiol 2000; 528:305-15 [20491028]