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150 mM HCO_3^- - How Does the Pancreas Do It? Clues from Computer Modelling of the Duct Cell

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Summary

Cystic fibrosis (CF) takes its name from the pathological changes that occur in the pancreas. Cystic fibrosis transmembrane conductance regulator (CFTR) is highly expressed in the pancreatic ductal epithelium and plays a key role in ductal HCO_3^- secretion. In humans, the pancreatic duct secretes near isotonic $NaHCO_3$. Experimental data suggests that HCO_3^- secretion occurs via apical Cl^-/HCO_3^- exchangers working in parallel with Cl^- channels (CFTR and calcium activated chloride channels, CaCC). Programming the currently available experimental data into our computer model [1, 2] (based on network thermodynamics) shows that while the anion exchanger/Cl^- channel mechanism will produce a relatively large volume of a HCO_3^--rich fluid, it can only raise the luminal HCO_3^- concentration up to about 70 mM. To achieve secretion of about 150 mM $NaHCO_3$ it is necessary to modulate the properties of the apical membrane transporters as the secreted fluid flows down the ductal system. On the basis of our computer simulations, we propose that HCO_3^- secretion occurs mainly via the exchanger in duct segments near the acini (luminal HCO_3^- concentration up to about 70 mM), but mainly via channels further down the ductal tree (raising luminal HCO_3^- to about 150 mM). We speculate that the switch between these two secretory mechanisms is controlled by a series of luminal signals (e.g. pH, HCO_3^- concentration) acting on the apical membrane transporters in the duct cell.

The pancreatic duct cell is a particularly interesting system in which to study HCO_3^- transport because it secretes HCO_3^- at high concentration. The maximum HCO_3^- concentration depends on the species, and varies between about 70 mM in the rat and 145 mM in cat, dog and humans [3, 4]. On the basis of studies that have been performed largely (but not exclusively) on ducts isolated from the rat and guinea pig pancreas the HCO_3^- secretory model shown in Figure 1 has been proposed [3, 4].

The key problem with this model is how the anion exchanger/Cl^- channel mechanism could secrete near isotonic $NaHCO_3$. Under these conditions the concentration of Cl^- in the luminal fluid will be very low (about 10 mM). This means that given reasonable values for intracellular [HCO_3^-], the intracellular Cl^- concentration would have to be very low indeed (less than 1 mM) to drive HCO_3^- secretion on an apical Cl^-/HCO_3^- exchanger. This intuitive assessment is confirmed by simulation data from our computer model, summarised in Figure 2.

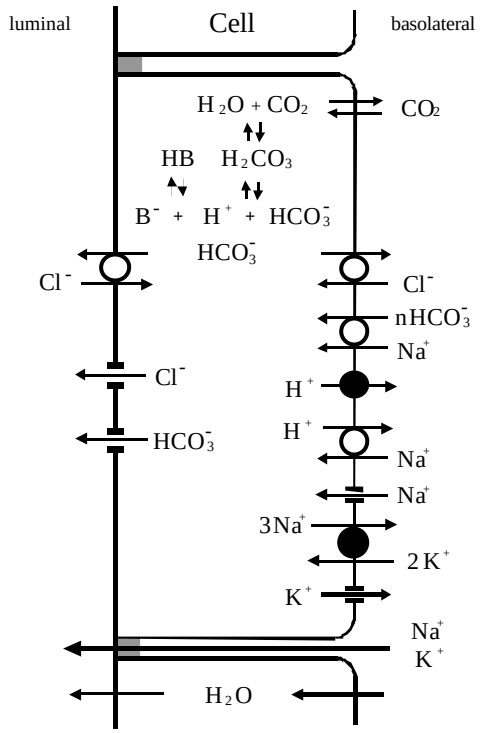

Figure 1. Schematic representation of the ion transport system in the pancreatic duct cell. (For illustrative purposes, the Cl^- and HCO_3^- conductances on the apical membrane are represented as separate pathways. In reality, these ions probably move through the same channels (CFTR and CaCC) as determined by their permeability characteristics. The model is based on data derived from experiments on rat and guinea pig duct cells.)

The computer simulation shows that the duct cell will secrete a fluid containing about 110 mM HCO_3^-, but only if the luminal HCO_3^- concentration is low (about 25 mM), as might be expected in the most proximal ducts just downstream from the acini (Figure 2). As the concentration of HCO_3^- in the duct lumen rises, the volume and HCO_3^- concentration of the secreted fluid decreases so that the maximal attainable HCO_3^- concentration in the luminal fluid is about 70 mM (Figure 2). The inability of this scheme to establish a luminal HCO_3^- concentration greater than 70 mM results from: i) a slowing of HCO_3^- efflux on the exchanger as luminal HCO_3^- concentration increases and luminal Cl^- concentration decreases (reversal of the exchanger will eventually occur), and ii) reduced HCO_3^- efflux via the conductive pathway as luminal HCO_3^- increases.

Thus while the cellular model shown in Figure 1 might describe how the rat pancreas works (maximum juice $[HCO_3^-]$ about 70 mM), it cannot explain how near isotonic HCO_3^- is

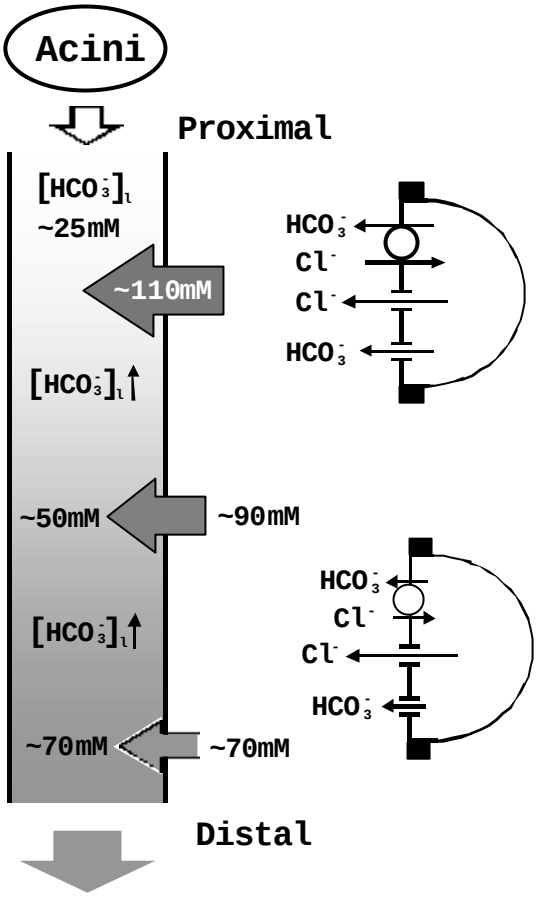

Figure 2. Computer simulation of pancreatic ductal HCO_3^- secretion using a simple Cl^- channel/anion exchanger mechanism. (We have used network thermodynamics to construct a three compartment mathematical model that simulates an epithelial cell *is situ* within an intact duct [2]. The model is based on published experimental data on the duct cell. It provides information about secretory rate and the HCO_3^- concentration in the secreted and luminal fluids, as well as transient and steady state data on intracellular pH and $[Cl^-]_i$, cell volume, membrane and transepithelial potentials, and the voltage divider ratio. The thickness of the arrows on the duct indicates the volume of fluid secreted at different levels in the ductal system. The length of the arrows on the cells indicates the activity of individual transport processes.)

secreted by species such as the guinea-pig, cat, dog and humans. It is possible that these species use a different HCO_3^- secretory mechanism at the apical membrane. However, in our view the evidence for this is not strong. Indeed, far from identifying differences between the rat and guinea pig duct cells, recent publications have emphasised the similarities in terms of the key transport elements that are involved in HCO_3^- secretion (see Figure 1).

Using our computer model we have investigated how the transport parameters on the duct cell must be altered for the scheme shown in Figure 1 to secrete near isotonic $NaHCO_3$. Based on the computer simulations, we propose a new hypothesis for pancreatic ductal HCO_3^- secretion. The hypothesis is summarised in Figure 3. We view the starting point of the HCO_3^- secretory process as the secretion (by the acini) of a small volume of a plasma-like fluid (containing 25 mM HCO_3^-) into the top of the ductal system. Programming the currently available experimental data into the computer model shows that the cell depicted in Figure 3 (top right) will produce a relatively large volume of secretion containing about 110 mM HCO_3^-. We call this cell the proximal cell, predict that it exists in the upper part of the ductal system close to the acini, and note that it represents one end of a functional spectrum within the ductal tree.

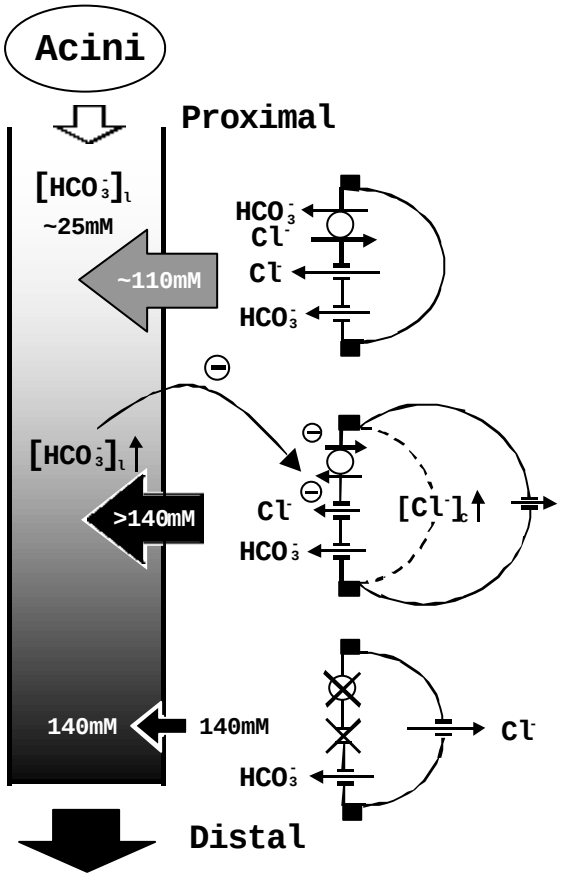

Figure 3. Computer generated hypothesis for near isotonic HCO_3^- secretion by the pancreatic ductal tree. (See text for details)

As the fluid flows along the first part of the ductal system, the luminal HCO_3^- concentration will rise and the net secretory flux of HCO_3^- from the proximal cell will fall (Figure 3). We propose that as this occurs, the activity of membrane transporters in the proximal cell is progressively modified to those of a cell that can maintain a small net secretory flux of HCO_3^- in the face of near isotonic $NaHCO_3$ in the lumen (Figure 3). We call this cell the distal cell and note that it represents the other end of the functional spectrum that exists in the ducts. Essentially, the high HCO_3^- secretion produced by the distal cells would dilute out the chloride in the secretions produced by acini and proximal cells in the upper regions of the ductal tree. Compared to proximal cells, the major changes in the transport parameters that allow the distal cells to secrete in the face of a high luminal HCO_3^- concentration are: i) a reduced luminal Cl^- permeability, ii) a reduced activity of the luminal Cl^-/HCO_3^- antiporter, and iii) activation of a basolateral volume-sensitive Cl^- conductance to offset cell swelling due to intracellular Cl^- accumulation. Thus HCO_3^- secretion would occur mainly by the exchangers in duct segments near the acini (proximal cells), but mainly via the channels further down the ductal tree (distal cells).

As yet, there is no experimental evidence for high HCO_3^- concentration or pH inhibiting the

anion exchanger on the apical membrane of the duct cell. However, some data does exist supporting other predictions made by the model. For example, we have shown that volume-activated Cl^- channels are present in the duct cell [5], and that an increase in extracellular [HCO_3^-] reduces the CFTR Cl^- conductance of guinea-pig duct cells [6]. The mechanism by which extracellular HCO_3^- inhibits CFTR is unknown. Both the Cl^- and HCO_3^- conductances on the luminal membrane probably reside within the same anion channels (i.e. CFTR and Ca^{2+}-activated Cl^- channels). This means that signals from the duct lumen would have to change the characteristics of ion permeation through these channels. This might come about from multi-ion characteristics. In this respect it is interesting to note that CFTR is known to be a multi-ion channel [7]. However, we should be clear that decreasing apical membrane Cl^- permeability does not imply a change in the anion selectivity of the duct cell luminal membrane. The necessity for decreasing apical membrane Cl^- permeability comes only from the requirement for a lower Cl^- as compared to HCO_3^- efflux across the luminal membrane at negative membrane potentials (about -50 mV), and not from a requirement to increase the anion selectivity of the apical membrane to HCO_3^- over Cl^-. Indeed, our patch-clamp study showed that high extracellular [HCO_3^-] did not change the Cl^-:HCO_3^- selectivity ratio of CFTR channels in guinea-pig pancreatic duct cells, although Cl^- efflux at negative membrane potentials was inhibited markedly [6]. It is, therefore, important to measure the ratio of Cl^- to HCO_3^- effluxes across the luminal membrane of the duct cell under defined experimental conditions and to compare those data with values predicted by the model.

Key words Cystic Fibrosis; Models, Theoretical; Pancreatic Ducts; Sodium Bicarbonate

Abbreviations CaCC: calcium activated chloride channels; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator

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