PERSPECTIVES

Keeping up with bicarbonate

Ivana Novak

August Krogh Institute, Copenhagen University, DK-2100 Copenhagen, Denmark

Pancreatic ducts secrete a HCO_3^--rich fluid that conveys an enzyme-rich fluid secreted by acini towards duodenum. This alkaline secretion is important for solubilisation of secreted enzymes and for neutralisation of acid chyme entering duodenum. Defects in ductal secretion underlie the pancreatic pathology that occurs in cystic fibrosis and certain forms of pancreatitis. The question of present interest is how the ductal epithelium secretes HCO_3^- at concentrations that are several-fold higher than in plasma. An apparent complicating factor is the observation that the rate of juice secretion and its HCO_3^- content vary among the species that we study. For example, rat pancreas produces 60–80 mM HCO_3^- and has modest secretory rates (5–10 μl (g min)$^{-1}$), while pancreas of cat, guinea-pig, pig and human is capable of secreting at higher rates (20–50 μl (g min)$^{-1}$) and produces a maximum of 120–150 mM HCO_3^-. Nevertheless, in all cases the concentration of HCO_3^- and its mirror companion, Cl^-, depend on secretory rates such that at high rates HCO_3^- is high and Cl^- is low, while at low rates the positions are interchanged.

Solving the question of how pancreatic ducts secrete HCO_3^- has been quite a challenging task, not least technically. Pancreatic ducts form only 2–4% of the total pancreatic tissue and the techniques of isolating and studying them are tedious. In this issue of *The Journal of Physiology*, Ishiguro *et al.* (2000) use perfused interlobular ducts (100–150 μm in diameter) from guinea-pig pancreas and pH_i measurements to study HCO_3^- permeability. In an extension of their previous studies they show that HCO_3^- enters duct cells across the basolateral membrane via a Na^+–HCO_3^- cotransporter (NBC), thought to transport 1 Na^+ for 2–3 HCO_3^-. Other studies show that HCO_3^- entry is also indirect, involving CO_2 permeation, hydration by the carbonic anhydrase to HCO_3^- and H^+, after which H^+ is extruded out of the cell by the Na^+–H^+ exchanger, and/or the H^+ pump. Since inhibition of carbonic anhydrase decreases HCO_3^- secretion by 60–80% in most species, and since other lipid-soluble buffers can substitute for HCO_3^-/CO_2, these two systems are important in secretion.

The subsequent transport of HCO_3^- across the luminal membrane is more problematic. In intact pancreas, HCO_3^- secretion depends to a large extent on Cl^-. Furthermore, in cystic fibrosis, which is a defect in cystic fibrosis transmembrane regulator (CFTR)-Cl^- channels, pancreatic HCO_3^- secretion is reduced. The working model for pancreatic duct transport linking the two anions was derived for the small predominantly intralobular ducts ($< 40\ \mu$m) from rat pancreas (Novak & Greger, 1988). Stimulation by secretin and other agonists leads to opening of Cl^- channels, seen as a 20-fold decrease in the fractional resistance of the luminal membrane (Novak & Greger, 1991). These are CFTR-Cl^- channels or Ca^{2+}-sensitive Cl^- channels in some species (Gray *et al.* 1988, 1994). Cl^- re-enters the cell via a Cl^-–HCO_3^- exchanger, which sends HCO_3^- out of the cell. Na^+ permeates paracellularly and $NaHCO_3$ secretion is followed by isotonic water transport. This experimental model predicted the 60–80 mM HCO_3^- secretion found in the rat pancreas. A decrease in luminal Cl^- and/or a reduction in Cl^-–HCO_3^- exchange might favour direct HCO_3^- efflux through CFTR-Cl^- channels or through unidentified, probably CFTR-dependent, HCO_3^- channels/transporters. This solution may be relevant for high HCO_3^- secretors such as the guinea-pig ducts, although the driving forces are unknown. Nevertheless, so far the compliment of transporters found is the same as in the rat ducts.

The last hurdle in HCO_3^- secretion is to keep HCO_3^- in the lumen. There is a large chemical and possibly electrical gradient pulling it back into the cell. The study of Ishiguro *et al.* (2000) on guinea-pig ducts gives a new impetus to the field. They show that changes in luminal HCO_3^- from 25 to 125 mM have no effect on pH_i other than that due to permeation of CO_2. Only at 145 mM HCO_3^- is there a small back-leak of HCO_3^- into the cell. In contrast, corresponding changes in the basolateral solutions have marked effects. Here, HCO_3^- uptake includes NBC, Na^+–H^+ exchange and in addition Cl^-–HCO_3^- exchange. Importantly, Cl^-–HCO_3^- exchange on the basolateral membrane is much more extensive than on the luminal membrane. Possibly the luminal Cl^-–HCO_3^- exchanger is inhibited by HCO_3^-, or it does not reverse until $HCO_3^- \approx 145$ mM. Interestingly, a recent study of O'Reilly *et al.* (2000) shows that extracellular HCO_3^- also inhibits CFTR-Cl^- conductance. This study and that of Ishiguro *et al.* indicate that the luminal membrane is relatively impermeable to HCO_3^- in comparison to the basolateral membrane. These studies were performed on unstimulated ducts, but preliminary experiments indicate similar results with forskolin-stimulated ducts. The interpretation is that luminal Cl^-–HCO_3^- exchange contributes very little to stimulated secretion. Rather, HCO_3^- secretion takes place through a high permeability HCO_3^- channel, which could secrete up to 125 mM HCO_3^-. Such a model has several requirements. First, given that intracellular HCO_3^- is 10–15 mM, the cell membrane voltage (V_m) must remain hyperpolarised at or below −60 mV even during stimulation, otherwise HCO_3^- would move into the cell. In rat pancreatic ducts, where we know that V_m depolarises from about −60 to −35 mV during stimulation (Novak & Greger, 1988, 1991), the HCO_3^- efflux through the channel would not work. On the other hand, if V_m stays hyperpolarised, the driving force for electrogenic NBC entry is minimal. A further important requirement for HCO_3^- secretion is a closure of the basolateral Cl^-–HCO_3^- exchanger. Electrophysiological recordings and pH_i measurements on stimulated guinea-pig ducts would test this theory, or reveal if we need to seek new solutions.

An alternative feasible interpretation of Ishiguro's data is that ducts can be shut off from secretion by a high luminal HCO_3^- and act more as Cl^-–HCO_3^- exchangers relying on HCO_3^- from hydration of CO_2 and exchanging luminal HCO_3^- for plasma Cl^-. In this context it is important to recall that the anion content of secretion changes with secretory rates, and in fact the main duct and possibly larger interlobular ducts can perform this exchange. We will need to find out whether these different transport modes are due to different functions of one duct, or whether the most proximal ducts form a HCO_3^--rich secretion that is modified during its passage through the ductal system and best accounts for excretory curves. A newly published theoretical model (Sohma *et al.* 2000) which proposes that the proximal moderately HCO_3^--rich secretion is somehow enriched by hypertonic HCO_3^- secretion distally seems a cumbersome solution. In either case, we will need to consider the morphology and function of the entire ductal tree. It is the intercalated and small intralobular ducts that are most numerous and rich in CFTR, carbonic anhydrase and other transport proteins. Interlobular ducts contain more cell types, are probably more electrically tight and may be more suited for modification of secreted fluid, as is the case in other exocrine glands. We may hope that future studies at the single cell level will be supplemented with comparative and integrative studies. Perhaps we may also settle the apparent species variations?

Gray, M. A. *et al.* (1988). *Journal of Membrane Biology* **105**, 131–142.

Gray, M. A. *et al.* (1994). *American Journal of Physiology* **266**, C213–221.

Ishiguro, H. *et al.* (2000). *Journal of Physiology* **528**, 305–315.

Novak, I. & Greger, R. (1988). *Pflügers Archiv* **411**, 546–553.

Novak, I. & Greger, R. (1991). *Pflügers Archiv* **419**, 76–83.

O'Reilly, C. M. *et al.* (2000). *Gastroenterology* **118**, 1187–1196.

Sohma, Y. *et al.* (2000). *Journal of Membrane Biology* **176**, 77–100.