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Primers on Molecular Pathways: Bicarbonate Transport by the Pancreas

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Abstract
The pancreas has both endocrine and exocrine functions. As an endocrine organ, stimulation of the pancreatic β-cells results in insulin secretion to control systemic glucose levels. The exocrine function of the pancreas and the need for alkaline pancreatic secretion (pH 8.0–8.5) have been appreciated for more than 40 years. Yet, our knowledge of the cellular mechanisms (signaling, transporters and channels) which accomplish these critical functions has evolved greatly. In the mid-1990s, basolateral Na-bicarbonate (HCO_3^-) uptake by NBCe1 (Slc4a4) was shown to be critical for the generation of approximately 75% of stimulated HCO_3^- secretion. In the last 10 years, several new HCO_3^- transporters in the Slc26 family and their interaction with the cystic fibrosis transmembrane conductance regulator-chloride channel have elucidated the HCO_3^- exit step at the ductal lumen. Most recently, both IRBIT (inositol 1,4,5-trisphosphate receptor-binding protein) and WNK [with no lysine (K)] kinase have been implicated as additional HCO_3^- secretory controllers.

The pancreas has both endocrine and exocrine functions. As an endocrine organ, stimulation of the pancreatic β-cells results in insulin secretion to control systemic glucose levels. The exocrine function of the pancreas and the need for alkaline pancreatic secretion have been appreciated for more than 40 years. Yet, our knowledge of the cellular mechanisms (signaling, transporters and channels) which accomplish these critical functions has evolved greatly.

The major function of the exocrine pancreas is to produce and secrete digestive enzymes into the duodenum. To prevent the pancreas from digesting itself, these digestive enzymes are kept inactive by elevated pH (>8.0) of the pancreatic juice. The high pH is the result of co-secretion of bicarbonate (HCO_3^-). That said, the HCO_3^- concentration of secretions varies greatly between species: approximately 70–75 mM for rats and mice, and about 150 mM (isotonic $NaHCO_3$) for cats, guinea pigs and humans. It is this difference in the 2-fold HCO_3^- concentration which has perplexed physiologists for 30 years. Reaching 75 mM $NaHCO_3$ can be accomplished by the channels and transporters shown in figure 1. However, additional HCO_3^- secretion must occur in the context of a continually decreasing ductal Cl^- concentration, which, based on the transport shown in figure 1, violates thermodynamics.

Pancreatic acinar cells are responsible for enzyme secretion while pancreatic ductal cells are responsible for

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the secretion of ions (predominantly Na^+ and HCO_3^-) and water. Failure in the transport of different ions including HCO_3^- in the pancreatic duct is most obvious in cystic fibrosis [mutations in the cystic fibrosis transmembrane conductance regulator (CFTR)], whereas in the pancreas the mucus secretion blocks the ducts [1].

In order to secrete HCO_3^-, ductal cells have HCO_3^- or H^+ transporters on both basolateral and apical membranes. Until recently, the major player in HCO_3^- secretion in the apical membrane was consider to be CFTR (fig. 1) [2–4]. At that time, CFTR was consider to be a chloride channel capable of HCO_3^- transport activated by an increase in intracellular cAMP [5, 6]. The model indicated that activity of carbonic anhydrase produces HCO_3^-, which is then secreted into the ductal lumen. At the same time, an H^+ is transported into the cell by a Na^+/H^+ exchanger, NHE3 (fig. 1). This model later evolved to include an apical HCO_3^- exchanger, presumed to be anion exchanger 1, 2 or 3 (Slc4a1–3)[1].

At the basolateral membrane, a HCO_3^- transporter was not initially proposed. A combination of CO_2 'diffusion across the membrane' and a Na^+/H^+ exchanger was sought and then shown to mediate net HCO_3^- entry [2, 7, 8]. The model indicated that activity of carbonic anhydrase produces HCO_3^-, which is then secreted into the ductal lumen. At the same time, an H^+ is transported into the cell by a Na^+/H^+ exchanger (fig. 1). Discovery [9] and cloning of the electrogenic $Na^+/nHCO_3^-$ co-transporter (NBCe1, Slc4a4) [10] revealed another basolateral entry pathway from the blood [8]. NBCe1 transports HCO_3^- into the ductal cells using the electrochemical gradients (Na^+, HCO_3^-, voltage) maintained by Na^+/K^+ ATPase. Thus, the Slc4 HCO_3^- transporters were believed to account for the major apical and basolateral transporters.

In 1999, Melvin and associates [11] discovered that Slc26a3 (downregulated in adenoma) functions as a Cl^--HCO_3^- exchanger. This discovery revealed that another protein family (Slc26) could have HCO_3^- transport activities, which has forever changed the dogma that only Slc4 proteins transport HCO_3^-.

With the discovery of Slc26 Cl^--HCO_3^- exchangers, the physiological role of CFTR-HCO_3^- transport by CFTR is becoming more disputed in the pancreatic ductal cells

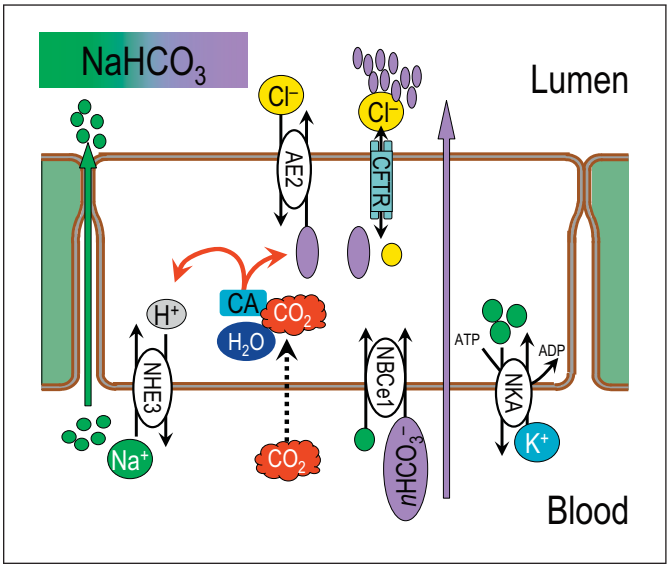

Fig. 1. HCO_3^- transport by the pancreatic duct, prior to 2004. Transductal $NaHCO_3$ secretion is accomplished by predominantly (75%) basolateral NBCe1-B and apical CFTR Cl^- channel. Other transporters involved in sodium, potassium and chloride are anion exchanger 2 (AE2) and NHE3. CO_2 from the blood is hydrated and then carbonic anhydrase (CA) makes HCO_3^- and H^+. For each H^+ excluded from the cell, 1 HCO_3^- is transported in the lumen of the pancreatic duct or exchanged for Cl^- on the basolateral membrane. NKA = Na^+/K^+ ATPase.

[12]. This dispute was further complicated by the discoveries that Cl^-:HCO_3^- coupling in mice is 2:1 for Slc26a3 [13] and 1:2 for Slc26a6 [13, 14]. A further feature is that human SLC26A6 coupling is 1:1 [15]. Three of 11 members of the Slc26 family seem involved in pancreatic HCO_3^- secretion (Slc26a3, Slc26a6 and Slc26a9) [13, 16, 17]. All are found on apical epithelial membranes [17, 18].

Slc26 proteins transport anions as exchangers or channels [18–21]. Slc26a6 also transports oxalate, formate and sulfate [14, 22, 23], whereas Slc26a9 possesses several transport modes: nCl^--HCO_3^- exchanger, anion channel [20, 24–26] and Na^+ transporter [26].

The relationship of Slc26 proteins with CFTR is a 2-way street. When R-CFTR binds the sulfate transporter anti-σ domain of Slc26a3 or Slc26a6, exchange activity is increased [13] while the same interaction with Slc26a9 inhibits transport [27]. This interaction also increases CFTR-Cl^- channel activity [16, 21], although Slc26a9-sulfate transporter anti-σ interaction activation requires cAMP [25, 28]. Not surprisingly, Slc26a9 does not stimulate ΔF508-CFTR activity [29], the most common mutation in cystic fibrosis, implying that Slc26a9

[1] SLC is the Human Genome Organization nomenclature for solute carriers. There are presently 46 known Slc gene families. Human genes are represented with capitals, while genes from other organism are given in lowercase letters. See http://www.bioparadigms.org/slc/menu.asp for detailed explanation of these Slc gene families.

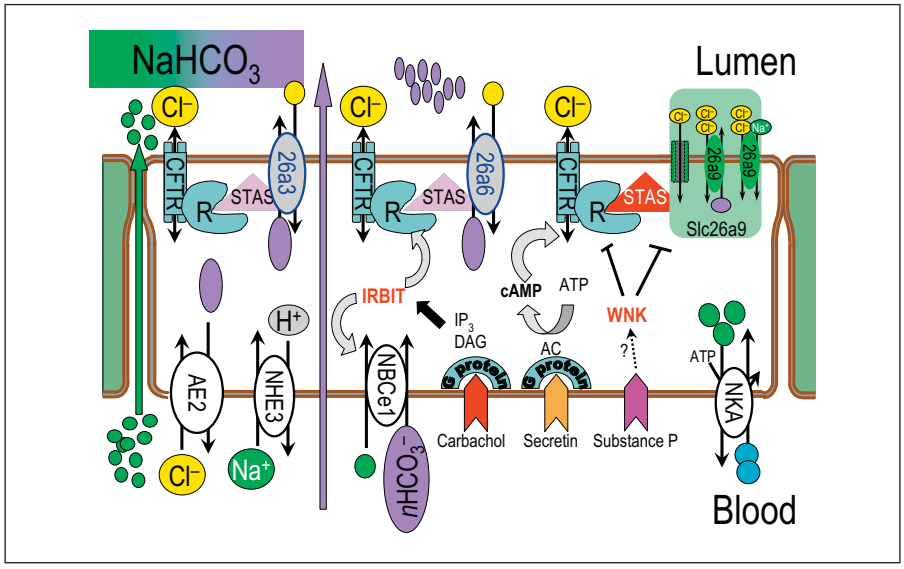

Fig. 2. An updated model of the HCO_3^- transport by the pancreatic duct. Apical players are Slc26a3, Slc26a6 and Slc26a9 members which can interact with CFTR. Slc26a9 and CFTR are inhibited by WNK kinases and possibly activated by substance P. NBCe1-B is the major basolateral HCO_3^- transporter (pNBCe1), which is activated by IRBIT, thereby increasing HCO_3^- secretion by pancreatic duct cells. R = R region; STAS = sulfate transporter anti-σ; AE2 = anion exchanger 2; IP_3 = inositol-3-phosphate; DAG = diacyl-glycerol; AC = adenylate cyclase; NKA = Na^+/K^+ ATPase.

may be associated with the severity of cystic fibrosis phenotypes.

Several of these interactions and regulatory cascades are controversial. Cl^- exit via CFTR and H^+ recirculating by NHE3 [30] are necessary for Cl^--HCO_3^- exchange activity of Slc26a3/Slc26a6 and HCO_3^- secretion. Slc26a6-like Cl^--HCO_3^- exchange activity increases with CFTR inhibition in the apical membrane of guinea pig pancreatic ductal cells [31], opposed to the results in HEK-293 cells [13]. Similarly, Slc26a9 [24] as well as CFTR [32] can be inhibited by WNK1/4 [with no lysine (K) 1/4] kinases, which are mutated in familial hyperkalemic hypertension. At physiological inositol-3-phosphate, its receptor releases IRBIT (inositol 1,4,5-trisphosphate receptor-binding protein) to apparently activate downstream molecules. Recently, IRBIT was found to increase basolateral NBCe1 [33, 34] and CFTR activity [34] (fig. 2). Thus, increasing IRBIT in pancreatic ductal cells would lead to stimulation of transductal HCO_3^- secretion [34].

Future studies will almost certainly reveal additional details of the regulation as well as the pathophysiology of pancreatic HCO_3^- secretion. Fortunately, we can now at least account for the mechanism of isotonic $NaHCO_3$ secretion found in humans.

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