INTRODUCTION: ACID-BASE FLUXES ALONG THE GASTROINTESTINAL TRACT

In multicellular organisms the digestive system exhibits marked acid/base segmentation and gradients across the epithelia. The most extreme examples of the acid/base transporters are the stomach and the pancreas, which conduct a vectorial transport of acid/base to one side and base/acid to the other side of the epithelium (Figure 1). In the stomach, the parietal cells of the pyloric glands secrete H⁺ toward lumen (HCl), leaving HCO₃⁻ to be transported into the interstitium and blood. Thus, the phenomenon of the alkaline tide, i.e., higher blood pH in connection with digestion, is well known as part of the post-prandial phenomenon of the alkaline tide, i.e., higher blood pH in connection with digestion, is well known as part of the post-prandial acid/base transport in pancreas—new challenges

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Along the gastrointestinal tract a number of epithelia contribute with acid or basic secretions in order to aid digestive processes. The stomach and pancreas are the most extreme examples of acid (H⁺) and base (HCO₃⁻) transporters, respectively. Nevertheless, they share the same challenges of transporting acid and bases across epithelia and effectively regulating their intracellular pH. In this review, we will make use of comparative physiology to enlighten the cellular mechanisms of pancreatic HCO₃⁻ and fluid secretion, which is still challenging physiologists. Some of the novel transporters to consider in pancreas are the proton pumps (H⁺-K⁺-ATPases), as well as the calcium-activated K⁺ and Cl⁻ channels, such as KCa3.1 and TMEM16A/ANO1. Local regulators, such as purinergic signaling, fine-tune, and coordinate pancreatic secretion. Lastly, we speculate whether dys-regulation of acid-base transport contributes to pancreatic diseases including cystic fibrosis, pancreatitis, and cancer.

Keywords: bicarbonate transport, proton transport, H⁺-K⁺-ATPase, KCa3.1, IK, TMEM16A, ANO1, pancreatic duct

Abbreviations: BK, big conductance K⁺ channel, also named KC₆.1 and maxi-K⁺, coded by KCNMA1; CaCC, Ca²⁺-activated Cl⁻ channel, e.g., TMEM16A also known as ANO1; CA, carbonic anhydrase; CCX, cholecystokinin, CF, cystic fibrosis; CaCC, Ca²⁺/Ca⁺1 activity; CFTR, the cystic fibrosis transmembrane conductance regulator; EBlO, 1-ethyl-2-benzimidazolone; GC, conductance for K⁺; H⁺-K⁺-ATPases or pumps, colonic type (coded by ATP12A) and gastric types (coded by ATP4A and ATP4B); IK, intermediate conductance K⁺ channel, also named KC₆.3.1; IRBIT, inositol 1,4,5-triphosphate (InsP3) receptor-binding protein released with InsP3; NBCe1 or pNBC, electrogenic Na⁺-Cl⁻ co-transporter; NBCe1, electroneutral Na⁺-Cl⁻ co-transporter; NHE, Na⁺/H⁺ exchanger; NKCC1, Na⁺-K⁺-2Cl⁻ cotransporter; P2X, protein kinase A; P2Y, proteins kinase C; SLC26A6, electrogenic Cl⁻/HCO₃⁻ exchanger; VNUT, vesicular nucleotide transporter, SLC17A9; V-H⁺-pump, vacuolar type H⁺-ATPase; ZG, zymogen granules.

Rune and Lassen, 1968; Wang et al., 2001; Niv and Fraser, 2002). In the intestinal phase of digestion, pancreatic ducts secrete HCO₃⁻-rich fluid that contributes to alkalinization of acid chyme in duodenum. The acid generated is then transported toward the interstitium, and one would expect an acid tide, depending on ingested food and passage through the stomach (Rune and Lassen, 1968; Ashley et al., 1994).

From these simple considerations several questions arise. Do the stomach and pancreas epithelia have some transport mechanisms in common, or do they solve the task of acid-base transport in different ways?

The molecular mechanism and regulation of stomach acid secretion is well established. In short, it involves gastric H⁺-K⁺-ATPases comprising of α1 and β subunits coded by ATP4A and ATP4B genes. These pumps are present in tubulovesicles of parietal cells and delivered to the luminal membranes in conjunction with specific K⁺ (KCNQ1, KCNJ15, KCNJ10) and Cl⁻ channels (CFTR, CLIC-6, SCL26A9), and thereby resulting in HCl secretion (Sachs et al., 2007; Forte and Zhu, 2010; Chu and Schubert, 2012). Gastric acid secretion is regulated by neural, hormonal, paracrine and chemical stimuli, e.g., acetylcholine, gastrin, ghrelin, histamine. As a protection against strong acid and pepsins, the surface epithelium secretes HCO₃⁻, mucus and other factors, forming gastric diffusion barrier (Figure 1A). The validity of the model is confirmed by well-used drugs, including proton pump inhibitors and H₂-histamine receptor blockers, to curb the
The latter two, which are expressed in pancreas (see below), other candidates remain to be explored.

Pancreatic ducts comprise 5–20% of the tissue mass, depending on the species; morphologically they are different—progressing from flat centroacinar cells, cuboidal cells in intercalated, and small intralobular ducts to columnar heterogeneous cells lining larger distal ducts (Kodama, 1983; Ashizawa et al., 1997; Bouwens and Pipeleers, 1998). At large, it is accepted that pancreatic ducts secrete isotonic NaHCO$_3$ rich fluid. However, the concentration of HCO$_3^-$ is not constant; it decreases with secretory rates—a pattern that is mirrored by Cl$^-$. The HCO$_3^-$ excretory patterns are remarkably similar between various species, providing that secretory rates are corrected for the duct mass (Figure 2A).

In early studies (Bro-Rasmussen et al., 1956), it was proposed that pancreatic secretion and ionic composition is a two stage process—primary secretion and ductal modification, the so called admixture hypothesis. Another, the exchange theory, also named the salvage mechanism, states that at lower secretory rates ductal transporters are presumably not saturated and therefore, are capable of exchanging luminal HCO$_3^-$ for interstitial Cl$^-$. This exchange phenomenon was first demonstrated on the main cat duct (Case et al., 1969). The third explanation, regarding varying HCO$_3^-$ concentrations, pertains H$^+$ secretion from acini (see above) or ducts (see below).

**NOVEL ION CHANNELS AND PUMPS CONTRIBUTING TO ACID-BASE TRANSPORT IN PANCREATIC DUCTS**

The ion transport models for pancreatic ducts have been described in several recent reviews (Steward et al., 2005; Steward and Ishiguro, 2009; Lee et al., 2012; Wilschanski and Novak, 2013). The outline of the model is given in Figure 2B. The following sections will focus on novel additions to the model.

**PROTON PUMPS**

Ion channels and transporters proposed in the classical model for HCO$_3^-$ secretion rely on gradients created by the Na$^+$/K$^+$-ATPase (Figure 2B). However, we cannot explain formation of high HCO$_3^-$ concentrations and the fact that inhibitors of NHE1, NBC (and NKCC1), and CA are relatively ineffective in blocking secretion (Grotmol et al., 1986; Fernandez-Salazar et al., 2004). One solution is that a primary pump could be involved, such as the vacuolar type H$^+$-ATPase (V-H$^+$-pump), to pump H$^+$ out to interstitium and leave HCO$_3^-$ for the luminal transport. In one study, such vacuolar H$^+$ pump on the basolateral membrane was proposed (Villanger et al., 1995) and detected immunohistochemically (Roussa et al., 2001). Several functional studies gave contradictory findings (Zhao et al., 1994; Ishiguro et al., 1997; de Ondarza and Hootman, 1997). Taking an inspiration from gastric glands, the colon and kidney distal tubules, we considered whether pancreatic ducts express H$^+$-K$^+$-ATPases. Indeed, we found that rodent ducts express both the gastric and non-gastric (colonic) types H$^+$-K$^+$-ATPases (Novak et al., 2011). Inhibition of these with proton pump inhibitors reduced pH$_2$ recovery in response to acid loads; more importantly, they reduced secretion in isolated pancreatic ducts. Thus, these functional studies support the theory that pancreatic ducts resemble gastric glands—just working in reverse, expelling H$^+$ toward the blood side.
and leaving HCO$_3^-$ for the luminal transport (Figure 1B). The immunohistochemical study showed that the H$^+$-K$^+$-ATPases (mainly colonic type) are localized to the basolateral membrane (Figure 2C).

However, H$^+$-K$^+$-ATPases, especially the gastric form, are also localized at or close to the luminal membrane (Figure 2C) (Novak et al., 2011). It seems counterintuitive to place H$^+$ pumps on the HCO$_3^-$ secreting luminal membrane. Nevertheless, there are epithelia that are high HCO$_3^-$ secretors and yet express H$^+$ pumps on the luminal membranes. For example, insect midgut and marine fish intestine have functional V-H$^+$-ATPase on the luminal membranes (Wieczorek et al., 2009; Wood et al., 2010; Guffey et al., 2011). Also other epithelia, which are not high HCO$_3^-$ secretors (HCO$_3^-$ < 25 mM), express various H$^+$ pumps on the luminal membranes. For example, airway epithelial transport both base and acid, and the airway fluid layer is slightly acidic (Fischer and Widdicombe, 2006). Some studies provide evidence for the presence of baflomycin A sensitive V-H$^+$ pump (Inglis et al., 2003; Fischer and Widdicombe, 2006; Shan et al., 2012); other studies show that transport is sensitive to SCH28080, an inhibitor of gastric (and also non-gastric) H$^+$-K$^+$ pumps (Smith and Welsh, 1993; Poulsen and Machen, 1996). The non-gastric, ouabain-sensitive H$^+$-K$^+$-pumps were also demonstrated in some studies (Coakley et al., 2003; Krouse et al., 2004; Shan et al., 2012).

Coming back to the pancreatic luminal H$^+$-K$^+$ pumps, let us speculate what their function may be. They could help to defend the cell against intracellular acidification, although there is a redundancy of acid/base transporters including several NHEs, NBCs, and Cl$^-$/HCO$_3^-$ exchangers (Figure 2B). Our proposal is—these luminal pumps are safeguarding luminal cell surface with acid secretions to protect against the bulk alkaline secretions, which at pH > 8 would be caustic to cells. Thus, pancreatic ducts would have protective buffer (and mucus) zone, which is reminiscent to the buffer zone in the stomach, though achieved by H$^+$ rather than HCO$_3^-$ secretion (Figures 1A,B). In addition, the luminal H$^+$-K$^+$ pumps would recirculate K$^+$ extruded by the luminal K$^+$ channels (Figure 2B). Lastly, luminal H$^+$-K$^+$ pumps in distal ducts would by virtue of H$^+$ secretion have more impact on pancreatic juice composition at low flow rates and minor at high flow rates, thus, explaining excretory curves for HCO$_3^-$ (Figure 2A).

**Ca$^{2+}$-ACTIVATED Cl$^-$ CHANNELS**

In addition to CFTR-dependent secretion, a number of studies showed that agonists acting via Ca$^{2+}$-signaling stimulate Ca$^{2+}$-activated Cl$^-$ channels (CaCC) and thus, could support duct secretion (Gray et al., 1989; Pahl and Novak, 1993; Winpenny et al., 1998; Szalmay et al., 2001; Pascua et al., 2009) (Figure 2B). The molecular identity of CaCC channels has been difficult to pinpoint [see (Duran et al., 2010)]. After suggestions of CCI-2 and bestrophins, the TMEM16/ANO family was discovered (Caputo et al., 2008; Schroder et al., 2008; Yang et al., 2008), and especially TMEM16A/ANO1 became a CaCC favorite. Recent studies show that human duct cell lines express TMEM16A, which re-localizes from cytosol to the luminal membrane upon purinergic stimulation and gives rise to secretory potentials (Wang and Novak, 2013; Wang et al., 2013). In human pancreatic samples immunohistochemistry shows TMEM16A in centro-acinar and small ducts cells (Bergmann et al., 2011).
It is relevant to ask whether TMEM16A and/or Ca\(^{2+}\) signaling pathways lead to HCO\(_3^-\) secretion. There are a few studies in support of this notion. For example, Ca\(^{2+}\) signaling via IRBIT stimulates NBCe1 (Shirakabe et al., 2006; Yang et al., 2009). A recent study on TMEM16A anion permeability shows that in HKE293 cell expression system and mouse salivary acinar cells the channel is directly modulated by calmodulin, which increases its HCO\(_3^-\) permeability (Jung et al., 2013). This is supported by a study on ex vivo salivary glands stimulated with acetycholine, which induced production of HCO\(_3^-\) rich pancreatic-like secretion when CI\(^-\) transport was inhibited (Novak and Young, 1986). Nevertheless, it cannot be excluded that there are other molecular candidates for CaCC, or that CFTR can convey part of the Ca\(^{2+}\)-activated Cl\(^-\) currents. The latter mechanism could involve Ca\(^{2+}\) sensitive adenylate cyclases and tyrosine kinases (Src2/Pyk complex), both of which could alter activity of CFTR, as shown for other epithelia (Billet and Hanrahan, 2013; Billet et al., 2013).

Another effect at the CFTR level could be priming of some PKC isoforms that enhance CFTR activity [see (Billet and Hanrahan, 2013)]. Lastly, it is highly unlikely that Ca\(^{2+}\) mediated signaling stands alone, rather the two major signaling pathways of Ca\(^{2+}\) and cAMP/PKA act synergistically in pancreatic ducts, e.g., via IRBIT regulation of CFTR and SLC26A6 (Park et al., 2013).

### K\(^+\) CHANNELS

The driving force for Cl\(^-\) or HCO\(_3^-\) exit is maintained by hyperpolarizing membrane potential created by opening of K\(^+\) channels, and G\(_e\) is both present on the basolateral and luminal membranes (Novak and Greger, 1988, 1991). Equivalent-circuit analysis has shown that stimulation of luminal K\(^+\) channels contributes with at least with 10% to the total conductance. Modeling in salivary glands confirms that such ratio of luminal to basolateral K\(^+\) channels would optimize secretion without destroying the transepithelial potential and transport (Cook and Young, 1989; Almassy et al., 2012). Furthermore, luminal K\(^+\) channels could contribute to secreted K\(^+\), as pancreatic juice contains 4–8 mM K\(^+\) (Sewell and Young, 1975; Cafilsch et al., 1979; Seow et al., 1991). The molecular identity of some K\(^+\) channels in pancreatic ducts is known, however, the exact localization and function remains to be verified [see (Hayashi and Novak, 2013)]. The K\(_{Ca}\).1.1 channels ( maxi-K, BK, coded by KCNM4) are present in pancreatic ducts (Hede et al., 2005; Venglovecz et al., 2011). The latter study proposes that these channels are expressed on the luminal membrane and activated by low concentrations of bile acids. However, earlier patch-clamp studies indicated that these channels were also located basolaterally (Gray et al., 1990; Hede et al., 1999). The K\(_{Ca}\).3.1 channel (IK, SK4, coded by KCNN4) was demonstrated in pancreatic ducts (Hede et al., 2005; Jung et al., 2006; Hayashi et al., 2012). Immunolocalization indicates that K\(_{Ca}\).3.1 is expressed on both membranes, though stronger on the luminal one (Figure 2B). Interestingly, the channel activator EBIO enhanced secretion potentials (Hayashi et al., 2012; Wang et al., 2013). Recent studies on pancreatic ducts offers molecular identities of several K\(^+\) channels, including KVLQT1, HERG, EAG2; Slick, and Slack (Hayashi et al., 2012), and interestingly the pH sensor TASK-2 (Fong et al., 2003). However, the function and regulation of these channels in pancreas physiology needs to be explored.

### PURINERGIC SIGNALING

Pancreatic secretion regulated by hormonal and neural systems is well documented (Lee et al., 2012; Wilschanski and Novak, 2013). Paracrine regulation is less explored, but it is highly relevant as it allows regulation within the gland and integration of acinar and ductal responses. Pancreatic ducts can be regulated by acinar factors (trypsin, guanylin, ATP) as well as retrograde factors (bile acids) (Kulaksiz et al., 2001; Alvarez et al., 2004; Venglovecz et al., 2008; Pallagi et al., 2011; Wang and Novak, 2013). Here we concentrate on purinergic signaling and present evidence that this signaling could fine-tune and coordinate pancreatic secretion on several fronts. Pancreatic ducts express several types of purinergic receptors including members from the G-protein coupled receptor families (adenosine, P2Y) and ligand-gated ion channels (P2X receptor) families (Novak, 2008, 2011) that can potentially stimulate a variety of intracellular signaling pathways (Burnstock, 2007; Surprenant and North, 2009; Lenertz et al., 2011; Wiley et al., 2011; Bilbao et al., 2012). These receptors regulate pancreatic duct ion transport, mucin secretion, and survival of fibroblastic pancreatic stellate cells (Jung et al., 2004; Haanes et al., 2012). ATP originates from ZG where it is accumulated by the vesicular nucleotide transporter VNUM (Haanes and Novak, 2010), and in addition ATP is presumably released by nerves and ductal epithelium (Bodin and Burnstock, 2001; Novak, 2011; Burnstock and Novak, 2012). Various ecto-nucleotidases are expressed and secreted, and potentially ATP/ADP and adenosine are effective regulators of ductal functions (Sørensen et al., 2003; Kittel et al., 2004; Yeugtkin et al., 2006; Burnstock and Novak, 2012).

ATP and UTP via P2 receptors have effects on intracellular Ca\(^{2+}\), intracellular pH, and transepithelial transport in both isolated ducts and in vivo pancreas (Ishiguro et al., 1999; Novak et al., 2010). The physiological response to nucleotides is side specific. Basolateral UTP inhibits secretion, most likely due to inhibition of K\(_{Ca}\).1.1 channels, presumably to prevent overextension of ducts. In contrast, luminal UTP/ATP application causes duct secretion and activation and Cl\(^-\) and K\(^+\) channels (Hede et al., 1999; Ishiguro et al., 1999; Wang et al., 2013). In particular K\(_{Ca}\).3.1 channel activation potentiates secretion (see above). It is well documented that purinergic receptor stimulation activates CFTR, Cl\(^-\)/HCO\(_3^-\) exchangers and TMEM16A on the luminal membrane (Namkung et al., 2003; Wang et al., 2013). Furthermore, P2 receptors activate CaCC and CFTR interdependently and synergistically, though exact receptors and signaling pathways remain to be elucidated (see above). In addition, some effects can be due to stimulation of A\(_{2A}\) and A\(_{2B}\) receptors, which stimulate CFTR (Novak et al., 2008).

A number of processes in purinergic signaling are pH sensitive, and this must be of relevance in pancreatic duct lumen. For example, nucleotidase activities, CD39 and CD73 types, are stimulated at alkaline pH 8–9 (Leal et al., 2005; Rucker et al., 2008), thus, favoring conversion of ATP to adenosine in duct lumen. Furthermore, purinergic receptors are also pH sensitive. From other preparations we know that extracellular acidification enhanced the potency of UTP up to 10 fold on the rat P2Y4 but not P2Y2 receptors (Wildman et al., 2003), and the P2X2 receptors was activated by acid pH (King et al., 1996). Extracellular alkalization enhances activity the P2X4 and P2X7 receptors (Clarke et al., 2000; Liu et al., 2009). Several types
of these receptors are expressed in duct lumen including the P2Y2 and P2X7 receptors, and these enhance pancreatic secretion and integrate acini-to-duct signaling (Novak, 2008; Novak et al., 2010).

SUMMARY AND PERSPECTIVES

The original cellular model for pancreatic HCO$_3^-$ secretion has been supplemented with molecular identities for many ion transporters/channels. The present review challenges present concepts by including active H$^+$ pumps in the model, and by comparing basic processes in pancreas and stomach. Furthermore, we present new additions to the model—Ca$^{2+}$-activated Cl$^-$ and K$^+$ channels, and propose that they work in synergy to regulate secretion. On the organ level, acini, and ducts integrate their function in acid/base transport and regulation, the latter exemplified by purinergic signaling. Further challenges lay in understanding dysregulation of acid-base transport in pancreas pathophysiology. In CF patients and animal models, pancreatic juice pH decreases from values >8.1 to <6.6, and pancreas contributes to duodenal hyperacidity (Freeman et al., 2001; Uc et al., 2011) [see (Wilschanski and Novak, 2013)]. It is not clear whether the problem relates to ductal and/or acinar secretion. In acute pancreatitis, which has complex etiologies, it is now appreciated that deficient pancreatic duct secretion can be the initiating factor (Lee et al., 2008; Hegyi et al., 2011b). Finally, in several cancer types, various acid-base transporters and associated ion channels, such as NHE1, NBCn1, CAIX, TMEM16A, K$_{10.1}$, and K$_{Ca3.1}$, change expression or function [see (Pedersen et al., 2013)]. Our knowledge about the role of acid-base transporters in pancreatic ductal adenocarcinoma clearly needs to be expanded, in order to provide potential diagnostic and therapeutic approaches.

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