PANCREAPEDIA
Exocrine Pancreas Knowledge Base

APA AMERICAN PANCREATIC ASSOCIATION

Molecular Mechanisms of Pancreatic Bicarbonate Secretion

Ikhyun Jun[1], Min Goo Lee[1], Shmuel Muallem[2]

1Department of Pharmacology, Brain Korea 21 PLUS Project for Medical Sciences, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul 120-752, Korea.

2The Epithelial Signaling and Transport Section, Molecular Physiology and Therapeutics Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, 20892

e-mail: mlee@yuhs.ac, Shmeul.Muallem@nih.gov

Version 1.0, January 27, 2017 [DOI: 10.3998/panc.2017.01]

1. Introduction

The human exocrine pancreas secretes 1-2 liters of pancreatic juice per day. When stimulated, the pancreas secretes alkaline pancreatic juice containing copious amounts of bicarbonate (HCO_3^-) (23, 74). HCO_3^- plays essential roles in the digestive system. HCO_3^- determines the pH of bodily fluids as a major buffer system that guards against toxic pH fluctuations (116). HCO_3^- in pancreatic juice neutralizes gastric acid, and provides an optimal pH environment for digestive enzymes to function in the duodenum (74). In addition, HCO_3^- acts as a moderate chaotropic ion that facilitates the solubilization of macromolecules, such as digestive enzymes and mucins (42). The importance of HCO_3^- is highlighted in the abnormal HCO_3^- secretion in cystic fibrosis (CF), which causes poor mucin hydration and solubilization leading to obstruction of ductal structures of the pancreas, intestine, vas deferens and the lung (112, 113).

The exocrine pancreas is composed of two major cell types, acinar and duct cells. Acinar cells secrete a small volume of isotonic, plasma-like, NaCl-rich fluid and digestive enzymes. Duct cells modify the ionic composition of the fluid and secrete the bulk of the fluid and $HCO_3^-$of the pancreatic juice. According to the Henderson-Hasselbalch equation, at pH 7.4 and 5% CO_2, the HCO_3^- equilibrium concentration in plasma is approximately 25 mM. In humans, dogs, cats, and guinea pigs, HCO_3^- concentration in postprandial pancreatic juice is higher than 140 mM (23, 74). This remarkable transport performance has attracted much attention from pancreatologists and physiologists. Current understanding of the molecular mechanism of pancreatic HCO_3^- secretion was improved by the recent identification of ion transporters and channels, including the cystic fibrosis transmembrane conductance regulator (CFTR) (61), the electrogenic Na^+-HCO_3^- co-transporter NBCe1-B (also known an pNBC1) (1), and the solute-linked carrier 26 (SLC26) transporters (25, 100), together with regulatory proteins, such as with-no-lysine kinase 1 (WNK1) (102), SPAK (30)and the inositol-1,4,5-triphosphate (IP_3) receptor binding protein released with IP_3 (IRBIT) (140).

2. Control of Pancreatic HCO_3^- Secretion

Pancreatic HCO_3^- secretion increases in response

to ingestion of a meal, and is regulated by multiple neurohumoral inputs. Fluid and enzyme secretion by acinar cells are controlled predominantly by an increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (88, 106, 107). Fluid and HCO_3^- secretion by duct cells are regulated by cAMP signals (74, 86) that synergizes with Ca^{2+} to generate the physiological response(105). Pancreatic ductal cells express receptors for a battery of hormones and neurotransmitters. The two major hormones controlling ductal fluid and HCO_3^- secretion are the G_s-coupled, cAMP generating hormone secretin and the G_q-coupled, Ca^{2+} mobilizing hormone cholecystokinin (CCK), which are released from neuroendocrine cells in the upper duodenum. Cholinergic vagal output via an enteropancreatic vagovagal reflex also plays an important role in controlling ductal fluid and HCO_3^- secretion. In addition to these classic stimulators, several other humoral agents are released by the pancreas for fine tuning its secretion, including insulin, somatostatin, purines, and prostaglandins (78). Additional information on hormonal control of pancreatic secretion can be found in a previous review (74) and the “Regulation of Pancreatic Secretion” section in Pancreapedia (18).

Humoral Control

***Secretin*:** The low pH (below 4.5) gastric chyme stimulates the release of secretin from duodenal S cells into the blood (12, 19). Secretin stimulates ductal fluid and HCO_3^- secretion and synergizes with Ca^{2+} mobilizing agonists to potentiate enzyme secretion by acinar cells. Plasma secretin levels rise after a meal (19, 110) and correlate with HCO_3^- output (118). Secretin-stimulated fluid and HCO_3^- secretion is modulated by both peptide hormones, such as CCK and somatostatin, and by vagal stimulation (38, 68, 144).

***CCK*:** CCK is a major stimulator of acinar cell enzyme and fluid secretion which is mediated by the Ca^{2+}-dependent exocytosis of zymogen granules and activation of apical (luminal) Cl^- channels, respectively. CCK also acts on pancreatic duct secretion; however, the effects of CCK on pancreatic duct differ among species. In humans, the effect of CCK alone on ductal fluid secretion is weak; however, CCK greatly potentiates the effects of secretin (144).

***Purines*:** Pancreatic duct cells express multiple purinergic receptor (P2Rs) types, including ionotropic P2X and metabotropic P2Y receptors at the apical and basolateral membranes (82). P2Rs are stimulated by purinergic ligands released from nerve terminals at the basolateral space, zymogen granules of acinar cells into the luminal space, or efflux by ductal ATP transporters to both the basal and luminal compartments. Stimulation of P2Rs increases $[Ca^{2+}]_i$ in duct cells (96, 98). Several studies have examined effects of P2Rs on ion transporters in ductal cell lines, but there are almost no studies on ductal fluid and HCO_3^- secretion. Ishiguro et al. demonstrated that luminal ATP stimulated, while basolateral ATP inhibited fluid and HCO_3^- secretion in guinea-pig pancreatic duct (51).

Neuronal Control

Pancreatic secretion is regulated by the enteric nervous system, which is composed of a gut-brain axis and an intrapancreatic system. The major neurotransmitter acting on pancreatic duct cells is acetylcholine released by vagal parasympathetic fibers. Duct cells express both M1 and M3 muscarinic receptors, which act through changes in $[Ca^{2+}]_i$, but the M3 receptors are likely the main receptors since their expression level is higher than the M1 receptors (31, 62). In humans, cholinergic stimulation enhances ductal secretion stimulated by secretin, likely by synergistic mechanism that is mediated by IRBIT (105). Vasoactive intestinal peptide (VIP) and ATP are also localized in parasympathetic nerve terminals (69, 97). Vagal stimulation causes VIP release that is coupled with fluid and HCO_3^- secretion (46, 69).

3. Key Transporters Involved in

Pancreatic HCO_3^- Secretion

Pancreatic HCO_3^- secretion is mediated by a coordinated function of transporters expressed in the apical and basolateral membranes of duct cells. Pancreatic HCO_3^- secretion can be divided into 2 steps. The first step is uptake of HCO_3^- into the duct cells from the blood through the basolateral membrane. The second step is efflux of HCO_3^- across the apical membrane of duct cells. Regulatory mechanisms in the cytosol that include ions like Cl^- and several kinases and phosphatases, act on the transporters to coordinate and integrate the secretory process. Recent advances in molecular, cellular, and physiological techniques have enhanced our understanding of the molecular identity, localization, function, and regulatory mechanisms of ductal ion transporters (75). The major ion transporters expressed in the apical and basolateral membranes of the pancreatic duct cells are summarized in **Table 1** and **Figure 1**.

Table 1: Transporters of pancreatic duct

Transporters in the luminal membrane of pancreatic duct.		
Transporters	**Gene**	**Function**
cAMP-activated Cl^- channel	CFTR (ABCC7)	Fluid and HCO_3^- secretion
Ca^{2+}-activated Cl^- channel	TMEM16/ANO family	Cl^- and HCO_3^-(?) secretion, lipids flipping
Anion exchangers	SLC26A3 (DRA/CLD)	HCO_3^- secretion, electrogenic Cl^-/HCO_3^- exchanger (Cl^-:HCO_3^- stoichiometry of 2:1 or higher)
	PAT1 (SLC26A6)	HCO_3^- secretion, electrogenic Cl^-/HCO_3^- exchanger (Cl^-:HCO_3^- stoichiometry of 1:2)
Na^+/H^+ exchangers	NHE3 (SLC9A3)	HCO_3^- reabsorption (HCO_3^- salvage mechanism)
	NHE2 (SLC9A2)	HCO_3^- reabsorption (?)
Na^+-HCO_3^- cotransporter	NBCn1-A (NBC3,, SLC4A7)	HCO_3^- reabsorption (HCO_3^- salvage mechanism)
K^+ channels	Maxi- K^+ channels (KCNMA1?)	Maintain membrane potential during stimulated secretion Modulate luminal HCO_3^- secretion
Water channel	Aquaporin 5 (AQP5)	H_2O flow
Transporters in the basolateral membrane of pancreatic duct.		
Transporters	**Gene**	**Function**
Na^+/H^+ exchangers	NHE1 (SLC9A1)	Na^+-coupled H^+ extrusion, pH_{in} homeostasis Contribute to basolateral HCO_3^- influx
	NHE4 (SLC9A4)	Role uncertain
Na^+-HCO_3^- cotransporters	NBCe1-B (pNBC1, SLC4A4)	Basolateral HCO_3^- entry
Anion exchangers	AE2 (SLC4A2)	pH_{in} homeostasis, Cl^-_{in} supplier (?)
Cation-chloride cotransporters	Na^+-K^+-$2Cl^-$ cotransporter (NKCC1, SLC12A2) K^+-Cl^- cotransporter (KCC1, SLC12A4)	Basolateral Cl^- uptake (in mouse and rat ducts, but not in guinea pig and human) Basolateral K^+ and Cl^- efflux Cell volume regulation
K^+ channels	Maxi- K^+ channels (KCNMA1)	Maintain membrane potential during stimulated secretion
	Small or intermediate	Maintain resting membrane potential

	conductance K^+ channels (KCNN4)	
Na^+, K^+-ATPase	Na^+, K^+-ATPase (ATP1B1-3)	Maintain inward Na^+ gradient and outward K^+ gradient that determines the membrane potential
Water channels	Aquaporin 1 (AQP1)	Water transport
	Aquaporin 5 (AQP5)	Water transport
Carbonic Anhydrases	CAXII	HCO_3^- supply to AE2 and NBCe1-B

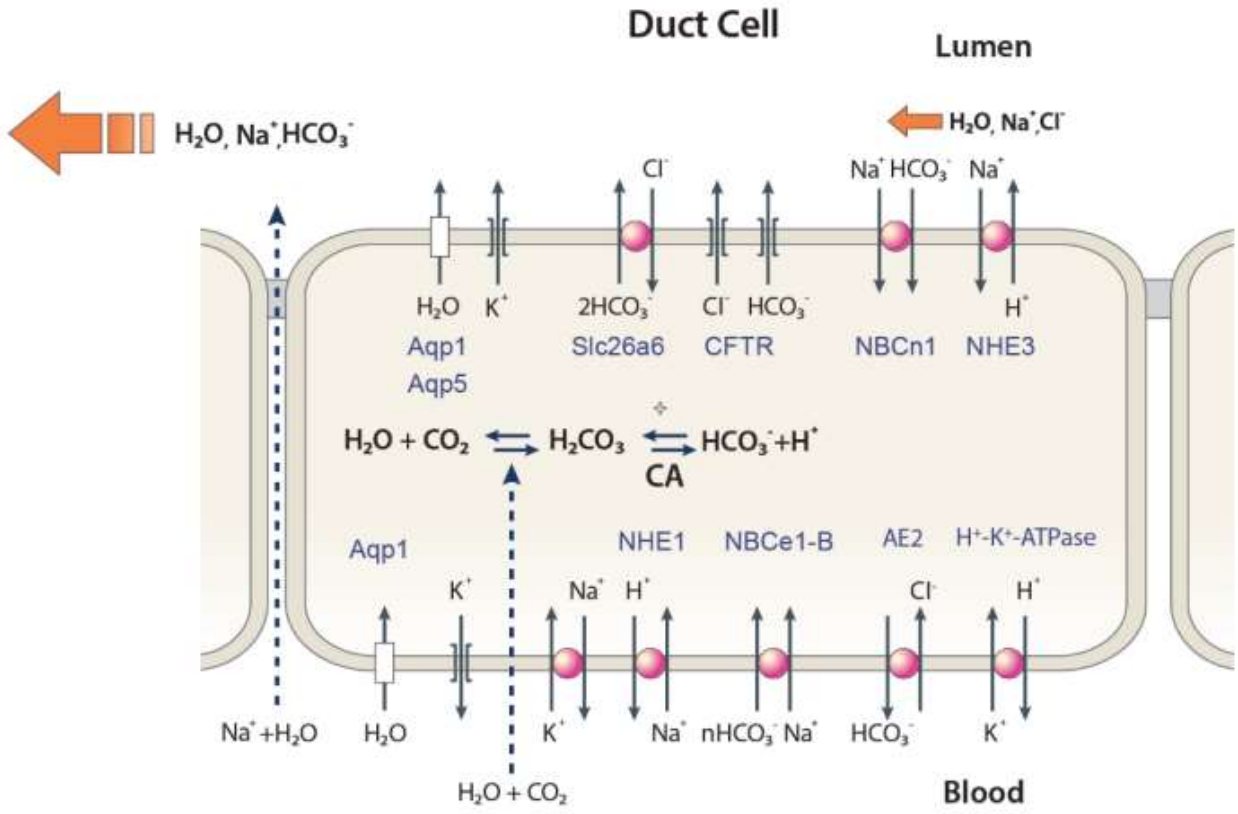

Figure 1. A schematic diagram depicting the transporters and channels in the apical (luminal) and basolateral membranes of pancreatic duct cells.
The main driving force for HCO_3^- secretion is achieved by the Na^+ gradient generated by the Na^+/K^+ ATPase pump and K^+ channels at the basolateral membrane, which generate the intracellular negative membrane potential. HCO_3^- is loaded mainly through the electrogenic ($1Na^+$-$2HCO_3^-$) NBCe1-B, and partly by NHE1 located in the basolateral membrane. Basolateral AE2 may act to supply Cl^-_{in} to maintain the secretion. Apical HCO_3^- secretion is performed by the interacting and functionally interrelated CFTR and Slc26a6. Transcellular HCO_3^- movement generates a lumen-negative electrical potential that results in paracellular Na^+ secretion through the paracellular pathway. Water follows Na^+ and HCO_3^- osmotically via paracellular and transcellular (aquaporins) pathways. In the resting state, luminal NHE3 and NBCn1-A function as salvage luminal HCO_3^-. Modified from (75).

Na^+/K^+ ATPase, and K^+ Channels

The main driving force for fluid secretion is achieved by the Na^+/K^+ ATPase pump and K^+ channels which generate the transmembrane Na^+ and K^+ gradients and the negative intracellular membrane potential (75, 101). The Na^+/K^+ ATPase pump is expressed in the basolateral membrane of the ducts (84, 117, 126, 131). The Na^+/K^+ ATPase pump generates the Na^+ and K^+ gradients by extruding 3 Na^+ ions in exchange for 2 extracellular K^+ ions which move inside using the energy of ATP hydrolysis. The K^+ gradient

generated by the pump, in conjunction with basolateral K^+ channels, yields a negative membrane potential. The Na^+ gradient is used to drive several Na^+-coupled solutes, including HCO_3^- absorption by the basolateral Na^+-HCO_3^- cotransporter NBCe1-B and basolateral and luminal Na^+/H^+ exchangers (NHEs). The negative membrane potential aids in HCO_3^- efflux through electrogenic transporters. MaxiK channels (KCNMA1) have been identified on the basolateral membrane of rat pancreatic duct cells, and are likely candidates for maintaining the membrane potential during agonist-stimulated HCO_3^- secretion (33). A Ba^{2+}-sensitive channel of 82 pS conductance (KCNN4) appears to be a basolateral K^+ channel, which is responsible for the resting K^+ permeability (99).

Na^+-HCO_3^- Co-transporters (NBCs)

The main ductal basolateral membrane HCO_3^- accumulation transporter is NBCe1-B (75). NBCe1-B was cloned from pancreas and was named pNBC1 (1). It was later re-named NBCe1-B as part of classification of the NBC family (11). NBCe1-B is an electrogenic transporter with a 1 Na^+: 2 HCO_3^- stoichiometry in pancreatic duct cells (37). NBCe1-B can be regulated by cAMP-dependent protein kinase A (PKA) phosphorylation at Ser1026 and Thr49 (36). In principle, Na^+/H^+ exchangers in the basolateral membrane (e.g. NHE1) can also mediate HCO_3^- influx in duct cells. However, the electrogenic NBCe1-B utilizes the Na^+ gradient more efficiently than the electroneutral NHE1 (1 Na^+: 1 HCO_3^-). Indeed, NBCe1-B contributes up to ~75% of the HCO_3^- influx during secretin-induced ductal fluid and HCO_3^- secretion in guinea pig (54, 56). The activity of NBCe1-B is controlled by multiple inputs, including IRBIT and the WNK/Ste20-related proline/alanine-rich kinase (SPAK) pathway (124, 141) and most notably intracellular Cl^- (121). In addition to NBCe1-B, the duct expresses electroneutral NBCn1-A (NBC3) on the apical (luminal) membrane (103, 111). This transporter may mediate HCO_3^- salvage in the resting state to maintain acidified pancreatic juice (32, 85).

CFTR

The discovery of acidic pancreatic juice in patients with cystic fibrosis (CF) was a milestone in understanding the mechanism of pancreatic HCO_3^- secretion (57). The CF transmembrane conductance regulator (CFTR) was discovered as the protein mutated in patients with CF (61, 114, 115). Although CFTR is a member of the ATP-binding cassette (ABC) transporters superfamily that usually act as membrane pumps that transport their substrates against the electrochemical gradient (20), CFTR functions as an anion (Cl^- and HCO_3^-) channel through which ions diffuse down the electrochemical gradient. CFTR is located at the apical membrane of pancreatic ducts (17, 129, 146) (and all secretory epithelia), and is activated by the cAMP/PKA pathway. At $[Cl^-]_i$ higher than 10 mM, CFTR functions as a Cl^- channel that has limited permeability to HCO_3^- (79, 109, 120). However, when $[Cl^-]_i$ drops to below 10 mM, CFTR anionic selectivity changes to increase HCO_3^- permeability and mediate luminal HCO_3^- exit (59, 102). Indeed, as has been shown in patients with CF (17, 50, 129), CFTR is critically involved in epithelial HCO_3^- secretion. This leads to revision of the original model of ductal HCO_3^- secretion, in which Cl^-/HCO_3^- exchangers mediate apical HCO_3^- efflux and CFTR facilitates the apical Cl^-/HCO_3^- exchangers by recycling the Cl^- (9). This continues to be the case at high Cl^-_{in}. However, at low $[Cl^-]_i$, HCO_3^- efflux via CFTR driven by the membrane potential has essential role in HCO_3^- efflux and HCO_3^--driven fluid secretion in the pancreatic duct (55, 128). Interestingly, this indicates that HCO_3^- permeability of CFTR is not fixed but is dynamically modulated by the protein kinase WNK1 (102). The low $[Cl^-]_i$ present during active HCO_3^- secretion triggers the activation of WNK1 and, in turn, WNK1 acts on CFTR to increase CFTR HCO_3^- permeability (102). Notably, CFTR mutations with altered WNK1-mediated

increase in HCO_3^- permeability are associated with chronic pancreatitis in humans (71).

CFTR has a more global role in ductal fluid and HCO_3^- secretion. In addition to functioning as a Cl^- and HCO_3^- channel, CFTR functions as a scaffold forming macromolecular complexes with other transporters and regulatory proteins at the apical membrane (75). CFTR has a PSD95/Discs-large/ZO-1 (PDZ) ligand at the C-terminus and binds to PDZ domains of adapter proteins, such as Na^+/H^+ exchanger regulatory factors (NHERFs) and SH3 and multiple ankyrin repeat domains 2 (Shank2) (72, 125) through which CFTR interacts and regulates the activity of slc26a6, slc26a3 (66), NHE3 (3) and NBCn1-A (103). Another interaction of CFTR is with soluble NSF attachment protein receptor (SNARE) proteins, A-kinase anchor proteins (AKAPs), kinases and phosphatases (39) that may serve to regulate CFTR activity and the activity of the transporters interacting with CFTR.

Cl^-/HCO_3^- Exchangers

Cl^-/HCO_3^- exchangers mediate the bulk of HCO_3^- exit across the apical membranes of the pancreatic duct cells until that the last critical portion of HCO_3^- exit is mediated by CFTR once it gains HCO_3^- permeability. In humans, members of the solute-linked carrier 4 (SLC4) and SLC26 families function as Cl^-/HCO_3^- exchangers. Among the SLC4 transporters, duct cells express AE2 (SLC4A2) at the basolateral membrane that regulates pH_i and protects against alkaline load (101). However, our recent studies revealed essential role for AE2 in ductal fluid and HCO_3^- secretion (47). Intuitively, basolateral HCO_3^- efflux mechanism should inhibit rather than stimulate ductal HCO_3^- secretion. It is not clear why AE2 is essential for ductal fluid secretion, but one possibility is that AE2 may provide the duct with Cl^- that is needed to keep the luminal slc26a6 functioning in a face of limited Cl^- provided by acinar secretion (47).

Among the SLC26 family transporters, SLC26A3, and SLC26A6 are located on the apical membrane of the pancreatic duct cells and mediate Cl^-/HCO_3^- exchange. Interestingly SLC26A3 has a $2Cl^-/1HCO_3^-$ stoichiometry (67, 122), while SLC26A6 functions as a $2HCO_3^-/1Cl^-$ exchanger (63, 122). A persistent osmotic gradient is needed to support the copious fluid secretion by the pancreatic duct. This is satisfied by the coupled action of NBCe1-B and SLC26A6 that results in a continuous net HCO_3^- (osmolite) transcellular transport and thus transcellular H_2O flow (123, 130, 136). In addition, SLC26 transporters interact with CFTR through the sulfate transporter and anti-sigma factor antagonist (STAS) domain, and regulate pancreatic secretion by activating CFTR (67).

Other Transporters, Channels, and Carbonic Anhydrases

***Na^+/H^+ exchangers (NHEs)*:** The SLC9A NHEs family is electroneutral $1Na^+/1H^+$ exchangers. The ubiquitous NHE1 (SLC9A1) is essential for pH_i homeostasis and supply Na^+ to the Na^+/K^+ ATPase pump (147), including the basolateral membrane of pancreatic duct. Diffusion of CO_2 from the blood into the duct and CO_2 generated by metabolism is hydrated by the action of carbonic anhydrases to generate HCO_3^- and H^+. NHE1 does not have a major role in basolateral HCO_3^- influx as revealed by minimal inhibition of fluid and HCO_3^- secretion by inhibition of NHE1 in pancreatic duct of most species (133, 139). Interestingly, the NHE3 isoform is expressed in the apical membrane of pancreatic duct and is thought to mediate HCO_3^- salvage at the resting state (73). At the resting state, the pancreatic juice is acidic, indicating an active H^+ secretion (32, 85) that may be mediated by the combined action of NHE3 and NBCn1-A. Similar to NBCn1-A (103), NHE3 interacts with CFTR via PDZ domain containing proteins (3), and is regulated by IRBIT (43, 44).

***Ca^{2+}-activated Cl^- channels (CaCCs)*:** Several members of the anoctamin (TMEM16/ANO) family

function as CaCC (15, 119, 143). TMEM16A/ANO1, TMEM16B/ANO2, TMEM16F/ANO6, and TMEM16K/ANO10 are expressed in pancreas (75). However, ANO1 is expressed in acinar but not duct cells (119), ANO6 functions as a flipase (131). The function of ANO2 and ANO10 in the pancreas is not clear at this time. Nevertheless, ample evidence show that the pancreatic duct (and ducts of other secretory glands) has CaCC activity in the apical membrane (34, 35, 134, 145). The molecular identity of this channel is not known at present, nor its function in HCO_3^- secretion. Several other CaCCs are known and are candidates for the ductal CaCC. In pancreatic acinar cells and other serous cells, ANO1 may have a role in HCO_3^- transport. At physiological $[Ca^{2+}]_i$ concentrations ANO1 functions as a Cl^- channel. However, at high $[Ca^{2+}]_i$ and perhaps at high $[Ca^{2+}]_i$ microdomains, ANO1 HCO_3^- permeability is increased by Ca^{2+}/calmodulin (58, 60), raising the possibility that ANO1 can provide an alternative Cl^- and HCO_3^- conduction in acinar cells.

***Aquaporins*:** Although the paracellular pathway is permeable to H_2O, H_2O flows, at least in part, transcellularly via the water channels aquaporins (AQP) family. This is best illustrated in salivary glands, where knockout of AQP5 markedly reduces salivation (83). Among the 13 AQPs, AQP1 and AQP5 are the major aquaporins in pancreatic duct (14, 64, 65). The role of individual aquaporins in the duct has not been established yet.

***Carbonic Anhydrases*:** A topic poorly studied that deserve more attention is the role of the ductal carbonic anhydrases (CAs) in fluid and electrolyte secretion, in particular with the emerging secretory epithelial diseases due to mutations in CAs. Mutations that affect the action of CA4 cause retinitis pigmentosa (5) and a mutation in CA12 causes salt wasting (28, 90). All transporters involved in fluid and HCO_3^- secretion are affected by the HCO_3^- concentration at the cellular compartments and microdomains that determine HCO_3^- availability at plasma membrane inner and outer surfaces. Hydration of CO_2 by CAs determines local HCO_3^- concentration both at the outer and inner plasma membrane surfaces (87). Several CAs are localized in the cytoplasm (such as CA2 and CA7) and several are anchored at the plasma membrane (such as CA4, CA12 and CA14) with the catalytic site at the extracellular surface and regulate HCO_3^- concentration at the basolateral (CA4 and CA12), or the luminal (CA4) membrane surfaces (29).

CAs localized in the plasma membrane and cytoplasm interact with H^+ and HCO_3^- transporters that mediates ductal fluid and HCO_3^- secretion and regulate their activity. CA4 interacts with the C terminus of NBCe1-A to increase its activity (4). The C terminus of NBCe1-A and NBCe1-B are conserved and thus likely CA4 regulates NBCe1-B. NBCn1-A recruits the cytoplasmic CA2 to the plasma membrane, where CA2 increases the activity of NBCn1-A (81). CA2 is closely associated with NHE3 and increases NHE3 activity (70). CA2 interacts with apparently novel site at the C terminus of NHE1 to regulate NHE1 activity (77). CA2 was reported to interact with the C terminus of slc26a6 to increase its activity. However, the role of other CAs, in particular the plasma membrane anchored CAs, on the activity of the slc26a6 and other SLC26 transporters has not been investigated yet. Finally, CA2 also interacts with AQP1 to increase water flux by AQP1 by an unknown mechanism (135).

A particularly interesting CA is the basolateral membrane anchored CA12 with a catalytic site at the extracellular membrane surface. A human mutation in CA12(E143K) is the cause of an autosomal recessive form of salt wasting, which leads to hyponatremia with hyperkalemia, high sweat Cl^-, dehydration and failure to thrive. (27, 28, 90). A recent work to understand the cause of the disease established a prominent role for CA12 in ductal fluid and HCO_3^- secretion. Thus, CA12

increased, while CA12 (E143K) markedly reduced. ductal fluid secretion in isolated ducts and *in vivo*. This could be attributed to a potent stimulation of ductal and topically expressed AE2 by CA12. The E143K mutation is a folding mutation that resulted in retention of CA12(E143K) in the ER (47). How exactly CA12 with external catalytic site activates AE2 is not obvious. CA12 may clear the extruded HCO_3^- from the membrane surface to prevent its buildup at the mouth of the AE2. If this can be established, it will be a new mode of regulating HCO_3^- transporters by CAs.

4. Regulation and Mechanism of Pancreatic HCO_3^- Secretion

Intracellular Signaling Pathways: cAMP and Ca^{2+}

The cAMP/PKA pathway is central in inducing ductal HCO_3^- secretion. Secretin is the major hormone that activates the cAMP pathway. VIP also signals to increase cAMP via VIP receptors (VPAC1) (26, 132). At maximal receptor stimulation, the cAMP/PKA pathway can fully activate fluid and HCO_3^- secretion by activation of the apical CFTR and the basolateral Na^+-HCO_3^- cotransporter, NBCe1-B (142). However, at physiological conditions the cAMP/PKA pathway synergizes with the Ca^{2+} signaling pathway to activate the secretory process (see below).

Several agonists that act on the pancreatic duct engage the Ca^{2+} signaling pathway. These include CCK, cholinergic stimuli, P2Rs, and protease-activated receptor 2 (PAR2) receptors (62, 107). Activated CCK and muscarinic receptors activate PLC to generate IP_3 that releases Ca^{2+} from intracellular stores and activates the membrane Ca^{2+} influx channels, Orai1 and TRPC. P2Rs (82, 94) and PAR2 (6, 91, 93, 95) also act through activation of the Ca^{2+} signaling pathway.

At physiological stimulus intensity, the cAMP and Ca^{2+} signaling pathways synergize to activate ductal secretion (59). Early studies *in vivo* already noted the synergistic action of ductal stimuli. Application of secretin at a level observed in the postprandial state only produces modest HCO_3^- and fluid output (24, 40). Application of CCK and stimulation of M1 and M3 receptors markedly augmented secretin-stimulated pancreatic fluid secretion, although alone CCK and muscarinic stimulation have minimal effect on ductal secretion (74, 144). The molecular mechanism of synergism was resolved with the discovery of regulation of ductal secretion by IRBIT which is discussed below. The cAMP and Ca^{2+} signaling pathways crosstalk on several additional levels to modulate the activity of each (59, 104). cAMP/PKA phosphorylates IP_3R2 to augment Ca^{2+} release from the ER (13). Ca^{2+} influx through the Orai1 channels activates the Ca^{2+}-dependent adenylyl cyclase (AC) AC8 (137). Ca^{2+} can also activate the CFTR-dependent Cl^-/HCO_3^- exchanger in CAPAN-1 human pancreatic duct cells (92), which may involve activation by IRBIT.

Regulation by IRBIT

Activation of NBCe1-B, slc26a6, and CFTR: IRBIT was isolated as a protein that interacts with the IP_3 receptors (IP_3Rs) and it can be dissociated from the IP_3Rs by IP_3 (21). IRBIT competes with IP_3 for binding to the IP_3Rs (8) to inhibit Ca^{2+} release. In fact, the IP_3Rs appear to function as IRBIT buffers to prevent IRBIT access to many transporters and targets regulated by IRBIT (75). The C-terminal region of IRBIT family proteins shows ~ 50% homology with the ubiquitous housekeeping enzyme S-adenosyl-l-homocysteine hydrolase (AHCY), with IRBIT having additional N terminal sequence while it lacks the hydrolase activity (7). The main known domains of IRBIT are PP1 and calcineurin binding motif, a PEST domain, a coiled-coil domain, and a PDZ ligand at the end of C terminus (75).

IRBIT plays an important role in pancreatic ductal secretion by regulating multiple transporters and mediating the synergistic action of the cAMP/PKA and Ca^{2+} signaling pathways (**Figure 2**). Knockdown of IRBIT in ducts and knockout in mice modestly inhibit fully stimulated pancreatic duct fluid and HCO_3^- secretion (142), and eliminate the physiological synergistic action of the cAMP/PKA and Ca^{2+} signaling pathways (105). IRBIT accumulates at the apical pole where IP_3Rs are highly expressed, but it can be found all over the cell where IP_3Rs are present (76). A search for IRBIT binding proteins identified NBCe1-B as a binding partner, where IRBIT binds to the autoinhibitory domain N terminus of NBCe1-B to activate it by removing the autoinhibition (124). Subsequent detailed studies, in particular with the pancreatic duct revealed that IRBIT at the apical pole potently activates CFTR (140, 142), SLC26A6 (105), and possibly NHE3 (44). At the basal side, IRBIT regulates NBCe1-B (124, 140, 142). IRBIT activates the transporters by multiple mechanisms. First, IRBIT recruits protein phosphatase 1 (PP1) to the transporters to dephosphorylate NBCe1-B and CFTR at sites that are phosphorylated by the kinase SPAK activated

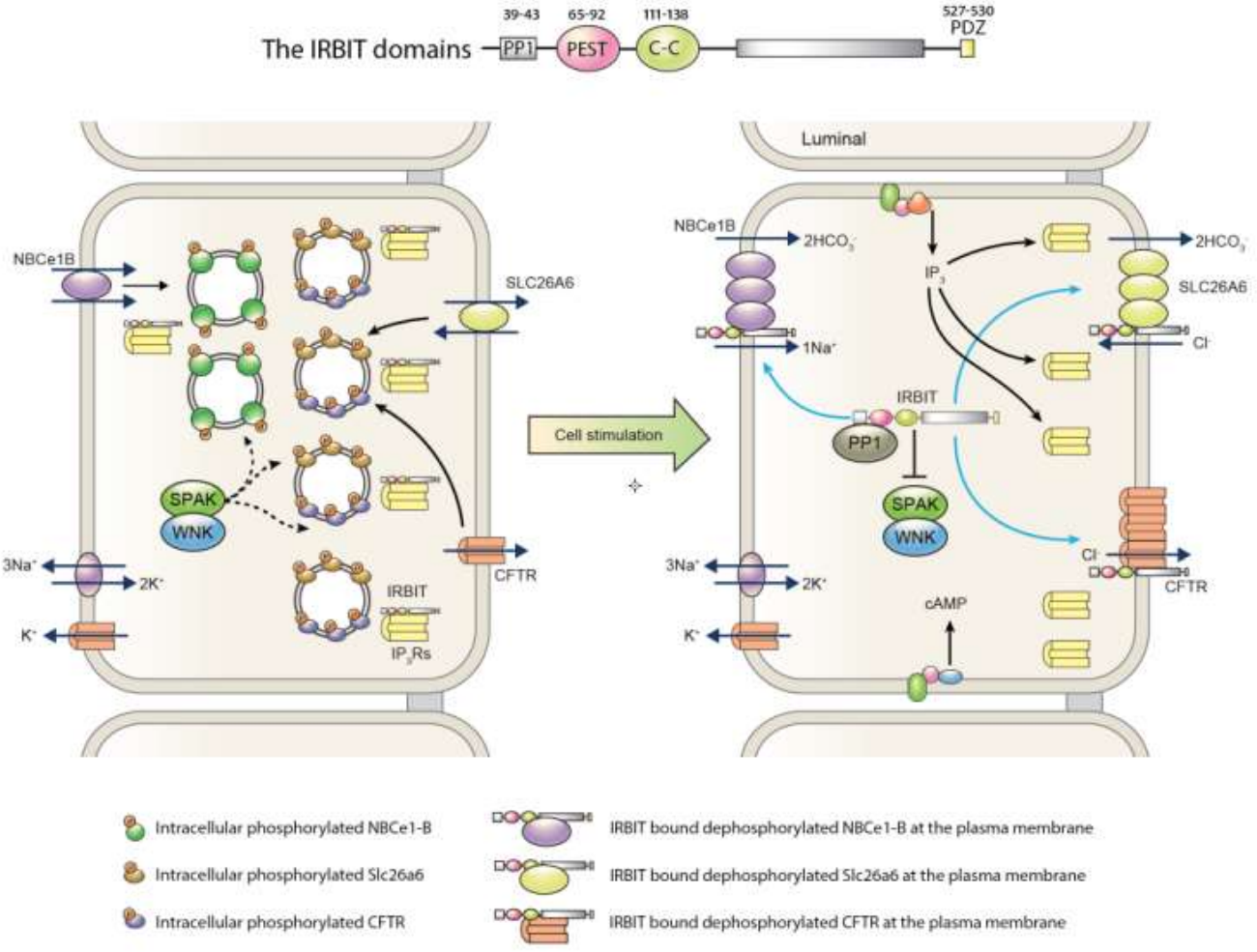

Figure 2. A model for IRBIT associated pathway of pancreatic ductal fluid and HCO_3^- secretion. Key domains of IRBIT related to HCO_3^- secretion are illustrated at the top of the figure. In the resting state, IRBIT is bound to IP_3Rs, and SPAK phosphorylates NBCe1-B, Slc26a6, and CFTR located at intracellular organelle. When the duct cells are stimulated, IP_3 is released and bound to IP_3Rs, while IRBIT is disengaged from IP_3Rs. PP1 recruited to IRBIT dephosphorylates transporters located at the plasma membrane. IRBIT also binds to the autoinhibitory domain of NBCe1-B to activate it. Increased surface expression of the transporters also aids pancreatic ductal HCO_3^- secretion. Modified from (104). See text for details.

by phosphorylation mediated by the kinases WNK1 and WNK4. This enhances the plasma membrane relocation of NBCe1-B, CFTR (140) and slc26a6 (105) from intracellular vesicular pools. At the plasma membrane, IRBIT directly interacts with the transporters to further increase their activity. The activation mechanism is not known in all cases. However, information exists for NBCe1-B in which IRBIT eliminates autoinhibition (124). The PDZ binding motif of IRBIT is required for assembling the IRBIT-NBCe1-B and IRBIT-CFTR complex (142).

***IRBIT and Synergism*:** An important action of IRBIT is mediating the synergistic action of the cAMP/PKA and Ca^{2+} signaling pathways (105) (see **Figure 2**). Physiological stimulus intensity must be quite weak to prevent cell toxicity that occurs under strong stimulation of all signaling pathways. Indeed, at physiological stimulus intensity the secretory process is activated only by about 10% or less of maximal stimulation. Synergism between weakly stimulated signaling is used to generate the maximal response while avoiding cell toxicity. IRBIT mediates the synergism between the cAMP/PKA and Ca^{2+} signaling pathways by translocation between cellular compartments and transporters. At the resting state, IRBIT is sequestered by the high level of IP_3Rs at the ductal ER apical pole and is not available for interaction with the transporters. The affinity of the IP_3Rs for IRBIT and IP_3 is regulated by PKA-mediated phosphorylation of specific IP_3Rs serine residues. Phosphorylation of the serine residues increases the affinity for IP_3 and at the same time decreases the affinity for IRBIT. Now, a small increase in IP_3 evoked by weak stimulation of the Ca^{2+} signaling pathway is sufficient to dissociate IRBIT from the IP_3Rs (105). The released IRBIT can bind to CFTR and slc26a6 first in intracellular vesicles to dephosphorylate them by the IRBIT-recruited PP1 (and perhaps calcineurin) and promote their translocation to the luminal membrane. At the luminal membrane, IRBIT activates the transporters to initiate ductal fluid and HCO_3^- secretion (105). Of note, the synergistic action of the cAMP/PKA and Ca^{2+} signaling pathways is eliminated by the knockout of IRBIT (105), highlighting the key role of IRBIT in the synergistic action of the cAMP/PKA and Ca^{2+} signaling pathways, which is the physiological way that ductal fluid and HCO_3^- secretion take place.

Regulation by $[Cl^-]_i$

***WNK1 and dynamic regulation of CFTR HCO_3^- permeability*:** The WNK proteins consist of four members (WNK1 – WNK4) with a conserved kinase domain that is noted for the unique position of the catalytic lysine residue (89). The discovery that mutations in WNK1 and WNK4 cause hypertension in humans has attracted much attention to the kinases function and regulation (138). The main function of the WNK kinases is the regulation of Na^+, K^+, Cl^-, HCO_3^-, and Ca^{2+} transporters in epithelia and brain (48, 49, 104). The WNKs act either by regulating surface expression of membrane transporters through modulation of their endocytosis or by phosphorylating the transporters and other target proteins directly or indirectly through affecting the effect of other kinases (49). Several functions of WNKs are mediated by phosphorylating and activating the downstream oxidative stress-responsive kinase 1 (OSR1) and SPAK (22). WNK1, WNK3, WNK4, SPAK, and OSR1 are expressed in the pancreatic duct (102, 140) and participate in the regulation of HCO_3^- transporters and channels (75). Accordingly, knockdown of WNK4 alone or a combined knockdown of WNK1, WNK3 and WNK4 increase pancreatic duct fluid secretion by removing a tonic negative effect of ductal HCO_3^- transporters (140). However, the role of the WNKs, in particular WNK1 changes at the terminal portion on the duct when $[Cl^-]_i$ is reduced to below 10 mM. WNK1, and perhaps the other WNKs, binds $[Cl^-]_i$ and its activity is regulated by $[Cl^-]_i$ (108).

The role of the WNKs and SPAK at $[Cl^-]_i$ above 10

mM is illustrated in the upper portion of the left model of **Figure 3**. The role of WNK1 in pancreatic HCO_3^- secretion at $[Cl^-]_i$ below 10 mM is illustrated in the bottom portion of the figure. Osmotic stress or low $[Cl^-]_i$ activates WNK1 (108). Notably, activation of WNK1 by low $[Cl^-]_i$ greatly increases the HCO_3^- permeability of CFTR (58, 102). During active pancreatic HCO_3^- secretion, Cl^- concentration in the pancreatic juice progressively reduces Cl^- absorption. Because of the low basolateral and high luminal Cl^- permeability (52, 102), $[Cl^-]_i$ rapidly decreases in response to the reduction in luminal duct Cl^- concentration. By the Nernst equation, at a

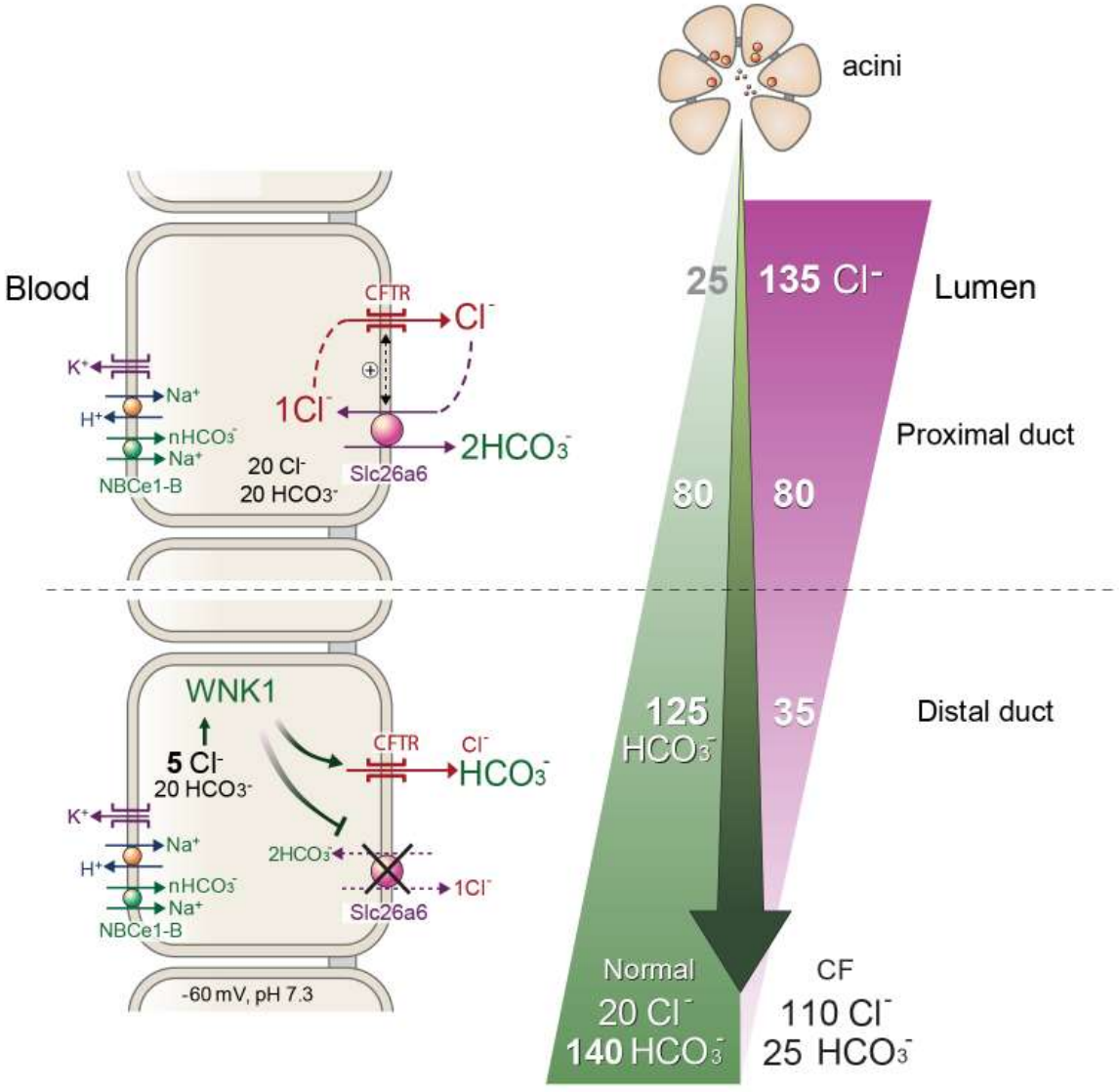

Figure 3. A model depicting WNK1-mediated regulation of CFTR in pancreatic ductal function. During active pancreatic HCO_3^- secretion, Cl^- concentration in the pancreatic juice is progressively reduced due to Cl^-/HCO_3^- exchange activities at the apical membrane of duct cells. Because the basolateral membrane of duct cells has poor Cl^- permeability but the apical Cl^- permeability is very high due to activation of CFTR, $[Cl^-]_i$ rapidly decreases in response to the reduction in luminal Cl^- concentration. Activation of WNK1 by low $[Cl^-]_i$ increases the P_{HCO3}/P_{Cl} of CFTR to over 1.0, which greatly augments HCO_3^- flux through the CFTR pore. Simultaneously, WNK1/SPAK pathway inhibits Slc26a6 to prevent HCO_3^- reabsorption. This mechanism enables an increase to as much as 140 mM HCO_3^- in pancreatic juice. See text for details. Modified from (75).

membrane potential of -60 mV, $[Cl^-]_i$ will be less than 1/10 of luminal Cl^- concentration. Indeed, ductal $[Cl^-]_i$ was estimated to be about 5 mM during cAMP-induced active secretion (52, 102).

WNK1 modulates the anion selectivity of CFTR by changing the pore size (58). Stimulation by WNK1 increases the pore size of CFTR from 4.8 Å to 5.3 Å, which facilitates the passage of larger anions, including HCO_3^- (4.3 Å, diameter), more than the smaller anion, Cl^- (3.7 Å, diameter) by reducing the energy barriers of size-exclusion, and ion dehydration. Changes in pore size affect the energy barrier of ion dehydration by altering the electric permittivity of the water-filled cavity in the pore. Dielectric constant (relative permitivity, ε) is a unit of electric permittivity, and the dielectric constant of water (ε_w) is approximately 80 at room temperature. Water molecules in confined geometries like ion channels exhibit a space-dependent reduction in the pore water ε_w down to 20, due to the restriction of the translational and rotational mobility of water molecules (2). Pore dilation relieves this restriction of water molecule movement and increases ε_w, which eventually leads to an increase in the overall ε of the anion channel pore. Indeed, the pore dilation induced by WNK1 activation increased the ε of the CFTR pore from 16 to 43 (58). In general, ions pass through the channel after dehydration (at least partial dehydration). Asymmetrically charged ions, such as HCO_3^-, show lower permeability than the symmetrically charged ions, such as Cl^-, due to the high hydration/dehydration energy barrier. The increase in anion channel pore ε greatly alleviates the dehydration penalty of the asymmetrically charged HCO_3^-, and increases P_{HCO3}/P_{Cl} (**Figure 4**).

Interestingly, activated WNK1 while shifting CFTR

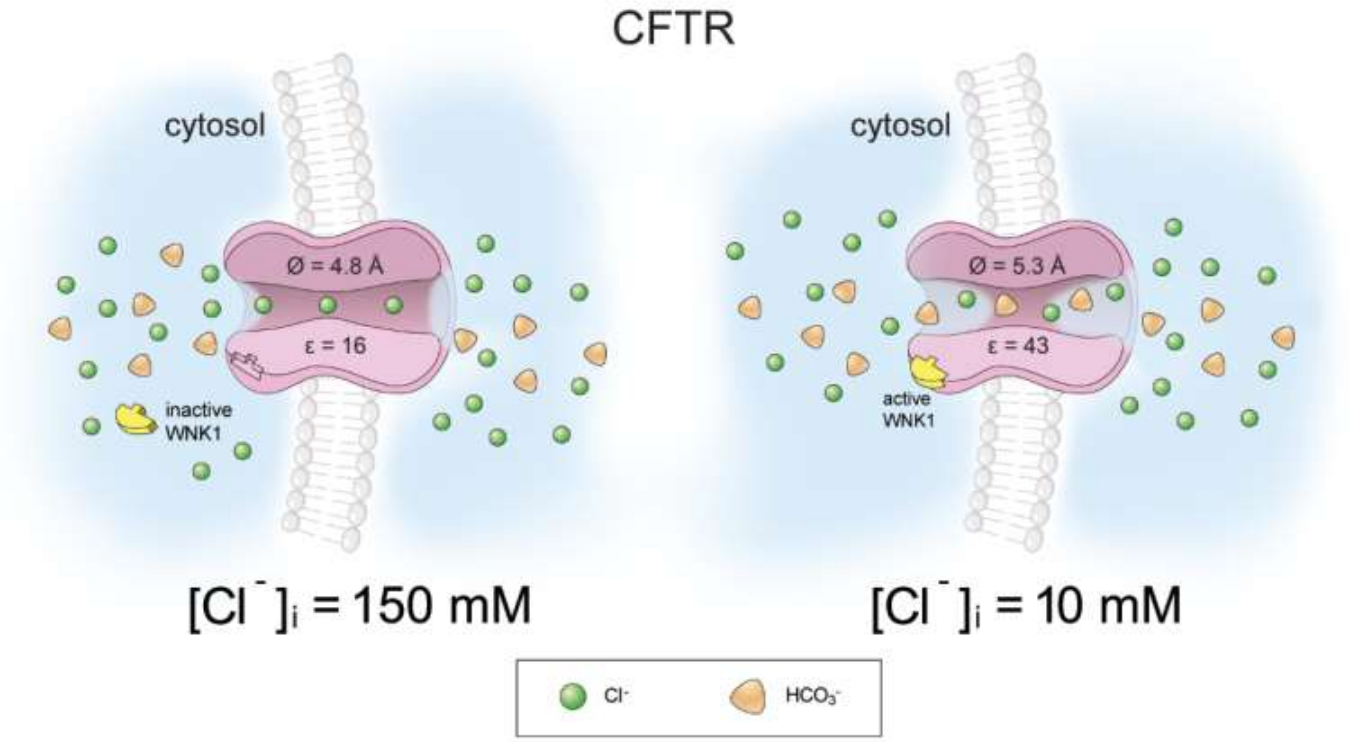

Figure 4. WNK1 modulates the anion selectivity of CFTR by changing the pore size. Stimulation by WNK1 increases the pore size of CFTR from 4.8 Å to 5.3 Å and the pore dilation increases the dielectric constant (ε) of the CFTR pore from 16 to 43. The increase in pore size facilitates the passage of the larger anion, HCO_3^- (4.3 Å, diameter), more than the smaller anion, Cl^- (3.7 Å, diameter) by reducing the energy barriers of size-exclusion. More importantly, the dielectric constant increase enhances the HCO_3^- permeability of CFTR by reducing energy barriers required for ion dehydration of HCO_3^- (58). See text for details.

anion selectivity, does not lose the inhibitory effect on SLC26A6 and SLC26A3 (102). When the luminal HCO_3^- concentration is greater than 140 mM, continuous activation of apical Cl^-/HCO_3^- exchange would reverse to absorb HCO_3^- from the lumen. This is more of a problem for the $2Cl^-/1HCO_3^-$ exchange by slc26a3 and less, if at all, for the $1Cl^-/2HCO_3^-$ slc26a6, especially at membrane potential of -60 mV across the luminal membrane. However, inhibition of the apical Cl^-/HCO_3^- exchangers is required to prevent the reverse mode of Cl^-/HCO_3^- exchange activity if slc26a3 dominates the exchange when ductal $[Cl^-]_i$ is below 10 mM and ultimately achieves HCO_3^- concentration above 140 mM in pancreatic juice (127, 129).

***Cl^-_{in} as a signaling ion*:** $[Cl^-]_i$ emerged as a signaling ion by regulating several HCO_3^- transporters. Regulation of the WNK kinases by $[Cl^-]_i$ and its role in CFTR HCO_3^- permeability was discussed above. By virtue of regulating the function of the WNK kinases $[Cl^-]_i$ may affect other transporters regulated by these kinases. A significant recent discovery is that $[Cl^-]_i$ profoundly regulates the activity of several Na^+-HCO_3^- cotransporters (NBCs) at the $[Cl^-]_i$ physiological range (121). $[Cl^-]_i$ regulates the activity of all NBCs tested NBCe1-B, NBCe1-C, and NBCe1-A. The IRBIT-independent activity of NBCe1-B is inhibited by $[Cl^-]_i$ between 60-140 mM that is outside the physiological range and may function to inhibit NBCe1-B activity under pathological conditions. Most notably, when activated by IRBIT, NBCe1-B activity is reduced by $[Cl^-]_i$ in the range of 5-20 mM, where at 20 mM $[Cl^-]_i$, NBCe1-B activity is reduced to the basal, IRBIT-independent level. Molecular analysis identified two Cl^- interacting motifs at the N terminus of NBCe1-B that mediate high and low affinity inhibition by $[Cl^-]_i$. Regulation of NBCe1-B is mediated by sites that contain the GXXXP motif. The first site mediates the high $[Cl^-]_i$ affinity (5-20 mM) regulation of NBCe1-B and the second site mediates the low $[Cl^-]_i$ affinity (60-140 mM) regulation of NBCe1-B (121). NBCe2-C activity is not regulated by IRBIT and in this case regulation of NBCe1-C is mediated by a single site containing the GXXXP motif and takes place at $[Cl^-]_i$ between 10-30 mM. Regulation of NBCe1-A by $[Cl^-]_i$ is mediated by a cryptic Cl^- interacting site containing the GXXXP motif. The cryptic NBCe1-A $[Cl^-]_i$ interacting sites was unmasked by deletion of residues 29-41.

Hence, cells have $[Cl^-]_i$ sensing mechanism that plays an important role in the regulation of Na^+ and HCO_3^- transporters that mediated the critical step of HCO_3^- influx in the process of ductal fluid and HCO_3^- secretion. At $[Cl^-]_i$ of up to 20 mM, CFTR functions mostly as a Cl^- channel and slc26a6 mediates most ductal HCO_3^- secretion. As $[Cl^-]_i$ is reduced below 20 mM and additional HCO_3^- secretion takes place in the face of unfavorable Cl^- and HCO_3^- gradients across the apical membrane, there is an increased demand for HCO_3^- entry across the basolateral membrane. Pancreatic duct cells achieve this by $[Cl^-]_i$-mediated regulation of NBCe1-B and CFTR, at which NBCe1-B activity and CFTR HCO_3^- permeability gradually increase as $[Cl^-]_i$ is reduced towards 5 mM.

A Model for Pancreatic HCO_3^- Secretion

Electrogenic HCO_3^- transporters can secrete higher concentrations of HCO_3^- than electroneutral transporters when the electrorepulsive force generated by the negative membrane potential is coupled to the efflux of HCO_3^-. The electrogenic SLC26A6 exchanger with the stoichiometry of 1 Cl^-: 2 HCO_3^-, can achieve luminal HCO_3^- concentration of up to about 120 mM at apical membrane potential of -60 mV (129). To drive luminal HCO_3^- concentration to 140 mM, the physiologic HCO_3^- concentrations in pancreatic juice, another mechanism is needed (129). Such a mechanism should be Cl^- independent, since significant fraction of pancreatic HCO_3^- secretion is retained in the absence of luminal Cl^- (53, 55). The WNK1

activated CFTR satisfy these requirements. At $HCO_{3\,in}^-$ in stimulated duct cells above 25 mM and membrane potential of -60 mV CFTR mediated HCO_3^- efflux even at luminal HCO_3^- concentrations of above 140 mM. The transcellular basal to luminal electrogenic HCO_3^- transport by both slc26a6 and CFTR generates a lumen-negative electrical potential that results in paracellular Na^+ secretion. Water flows down the osmotic gradient generated by the Na^+ and HCO_3^- fluxes via paracellular and transcellular (aquaporins) pathways. Overall, these processes generate an efficient mechanism for HCO_3^-–driven ductal fluid secretion to generate the volume and HCO_3^- content of the pancreatic juice.

5. Conclusions

The mechanism by which the human pancreatic duct secretes nearly isotonic HCO_3^- solution has long been an enigmatic question for both physiologists and clinicians (74, 129). When Bayliss and Starling first noticed that the exocrine pancreas secretes alkaline fluid, they assumed that carbonate is responsible for the strong alkalinity of the pancreatic juice (10). Later, with better understanding of the carbonate/HCO_3^-/CO_2 buffer system (45), it became clear that the exocrine pancreas secretes fluid in which the dominant anion is HCO_3^-, and HCO_3^- secretion is coupled to fluid secretion (16, 24, 41). Current understanding indicates that activation of three key transporters, the basolateral NBCe1-B (and likely AE2), and the luminal SLC26A6 and CFTR, and their synergistic regulation by the cAMP and Ca^{2+} signaling pathways through IRBIT and WNK1 perform for vectorial pancreatic HCO_3^- secretion that drives fluid secretion. NBCe1-B, with a 1 Na^+/2 HCO_3^- stoichiometry, is the main HCO_3^- concentrating transporter in the basolateral membrane, and can achieve the necessary HCO_3^- influx (1, 54, 148). Basolateral AE2 activity is also required to support ductal HCO_3^- fluid and HCO_3^- secretion, although AE2 exact role is not known at present. The electrogenic SLC26A6, with a 1 Cl^-/2 HCO_3^- stoichiometry is the major apical Cl^-/HCO_3^- exchanger, which mediates most HCO_3^- efflux in the early step of pancreatic HCO_3^- secretion (67, 80). Activated WNK1 increases HCO_3^- permeability of CFTR, allowing further apical HCO_3^- efflux and setting pancreatic juice HCO_3^- concentrations above 140 mM (102). Our understanding of the mechanism of pancreatic fluid and HCO_3^- secretion will continue to improve as our knowledge of existing pathways increases and new mechanisms are identified and delineated, to provide a better scientific basis for therapeutic approaches to treat diseases like cystic fibrosis and acute and chronic pancreatitis.

6. Acknowledgments

We thank Dong-Su Jang for assistance in illustrations. This work was supported by grant, 2013R1A3A2042197, from the National Research Foundation, the Ministry of Science, ICT & Future Planning, Republic of Korea and by Intramural NIH/NIDCR grant DE000735-06.

7. References

1. **Abuladze N, Lee I, Newman D, Hwang J, Boorer K, Pushkin A, et al.** Molecular cloning, chromosomal localization, tissue distribution, and functional expression of the human pancreatic sodium bicarbonate cotransporter. *J Biol Chem* 273(28): 17689-17695,1998. PMID: 9651366.
2. **Aguilella-Arzo M, Andrio A, Aguilella VM and Alcaraz A**. Dielectric saturation of water in a membrane protein channel. *Phys Chem Chem Phys* 11(2): 358-365,2009. PMID: 19088992.
3. **Ahn W, Kim KH, Lee JA, Kim JY, Choi JY, Moe OW, et al.** Regulatory interaction between the cystic fibrosis transmembrane conductance regulator and HCO_3^- salvage mechanisms in model systems and the mouse pancreatic duct. *J Biol Chem* 276(20): 17236-17243,2001. PMID: 11278980.
4. **Alvarez BV, Loiselle FB, Supuran CT, Schwartz GJ and Casey JR**. Direct extracellular interaction between carbonic anhydrase IV and the human NBC1 sodium/bicarbonate co-transporter. *Biochemistry* 42(42): 12321-12329,2003. PMID: 14567693.

5. **Alvarez BV, Vithana EN, Yang Z, Koh AH, Yeung K, Yong V, et al.** Identification and characterization of a novel mutation in the carbonic anhydrase IV gene that causes retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 48(8): 3459-3468,2007. PMID: 17652713.
6. **Alvarez C, Regan JP, Merianos D and Bass BL**. Protease-activated receptor-2 regulates bicarbonate secretion by pancreatic duct cells in vitro. *Surgery* 136(3): 669-676,2004. PMID: 15349117.
7. **Ando H, Kawaai K and Mikoshiba K**. IRBIT: a regulator of ion channels and ion transporters. *Biochim Biophys Acta* 1843(10): 2195-2204,2014. PMID: 24518248.
8. **Ando H, Mizutani A, Matsu-ura T and Mikoshiba K**. IRBIT, a novel inositol 1,4,5-trisphosphate (IP3) receptor-binding protein, is released from the IP3 receptor upon IP3 binding to the receptor. *J Biol Chem* 278(12): 10602-10612,2003. PMID: 12525476.
9. **Argent B and Case RM**. Pancreatic ducts. Cellular mechanism and control of bicarbonate secreation. *Physiology of the gastrointestinal tract*: 1473-1497,1994. PMID.
10. **Bayliss WM and Starling EH**. The mechanism of pancreatic secretion. *J Physiol* 28(5): 325-353,1902. PMID: 16992627.
11. **Boron WF, Chen L and Parker MD**. Modular structure of sodium-coupled bicarbonate transporters. *J Exp Biol* 212(Pt 11): 1697-1706,2009. PMID: 19448079.
12. **Brooks AM and Grossman MI**. Postprandial pH and neutralizing capacity of the proximal duodenum in dogs. *Gastroenterology* 59(1): 85-89,1970. PMID: 5426993.
13. **Bruce JI, Shuttleworth TJ, Giovannucci DR and Yule DI**. Phosphorylation of inositol 1,4,5-trisphosphate receptors in parotid acinar cells. A mechanism for the synergistic effects of cAMP on Ca^{2+} signaling. *J Biol Chem* 277(2): 1340-1348,2002. PMID: 11694504.
14. **Burghardt B, Elkaer ML, Kwon TH, Racz GZ, Varga G, Steward MC, et al.** Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human pancreas. *Gut* 52(7): 1008-1016,2003. PMID: 12801959.
15. **Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, et al.** TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Science* 322(5901): 590-594,2008. PMID: 18772398.
16. **Case RM, Harper AA and Scratcherd T**. The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat. *J Physiol* 201(2): 335-348,1969. PMID: 5780548.
17. **Catalan MA, Nakamoto T, Gonzalez-Begne M, Camden JM, Wall SM, Clarke LL, et al.** Cftr and ENaC ion channels mediate NaCl absorption in the mouse submandibular gland. *J Physiol* 588(Pt 4): 713-724,2010. PMID: 20026617.
18. **Chandra R and Liddle RA**. Regulation of Pancreatic Secretion. *The Pancreapedia: Exocrine Pancreas Knowledge Base*,2015. DOI 10.3998/panc.2015.38.
19. **Chey WY, Lee YH, Hendricks JG, Rhodes RA and Tai HH**. Plasma secretin concentrations in fasting and postprandial state in man. *Am J Dig Dis* 23(11): 981-988,1978. PMID: 31087.
20. **Deeley RG, Westlake C and Cole SP**. Transmembrane transport of endo- and xenobiotics by mammalian ATP-binding cassette multidrug resistance proteins. *Physiol Rev* 86(3): 849-899,2006. PMID: 16816140.
21. **Dekker JW, Budhia S, Angel NZ, Cooper BJ, Clark GJ, Hart DN, et al.** Identification of an S-adenosylhomocysteine hydrolase-like transcript induced during dendritic cell differentiation. *Immunogenetics* 53(12): 993-1001,2002. PMID: 11904675.
22. **Delpire E and Gagnon KB**. SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. *Biochem J* 409(2): 321-331,2008. PMID: 18092945.
23. **Domschke S, Domschke W, Rosch W, Konturek SJ, Sprugel W, Mitznegg P, et al.** Inhibition by somatostatin of secretin-stimulated pancreatic secretion in man: a study with pure pancreatic juice. *Scand J Gastroenterol* 12(1): 59-63,1977. PMID: 189382.
24. **Domschke S, Domschke W, Rosch W, Konturek SJ, Wunsch E and Demling L**. Bicarbonate and cyclic AMP content of pure human pancreatic juice in response to graded doses of synthetic secretin. *Gastroenterology* 70(4): 533-536,1976. PMID: 176080.
25. **Dorwart MR, Shcheynikov N, Yang D and Muallem S**. The solute carrier 26 family of proteins in epithelial ion transport. *Physiology (Bethesda)* 23: 104-114,2008. PMID: 18400693.
26. **Evans RL, Perrott MN, Lau KR and Case RM**. Elevation of intracellular cAMP by noradrenaline and vasoactive intestinal peptide in striated ducts isolated from the rabbit mandibular salivary gland. *Arch Oral Biol* 41(7): 689-694,1996. PMID: 9015570.
27. **Feinstein Y, Yerushalmi B, Loewenthal N, Alkrinawi S, Birk OS, Parvari R, et al.** Natural history and clinical manifestations of hyponatremia and hyperchlorhidrosis due to carbonic anhydrase XII deficiency. *Horm Res Paediatr* 81(5): 336-342,2014. PMID: 24714577.

28. **Feldshtein M, Elkrinawi S, Yerushalmi B, Marcus B, Vullo D, Romi H, et al.** Hyperchlorhidrosis caused by homozygous mutation in CA12, encoding carbonic anhydrase XII. *Am J Hum Genet* 87(5): 713-720,2010. PMID: 21035102.
29. **Frost SC**. Physiological functions of the alpha class of carbonic anhydrases. *Subcell Biochem* 75: 9-30,2014. PMID: 24146372.
30. **Gagnon KB and Delpire E**. Molecular physiology of SPAK and OSR1: two Ste20-related protein kinases regulating ion transport. *Physiol Rev* 92(4): 1577-1617,2012. PMID: 23073627.
31. **Gautam D, Han SJ, Heard TS, Cui Y, Miller G, Bloodworth L, et al.** Cholinergic stimulation of amylase secretion from pancreatic acinar cells studied with muscarinic acetylcholine receptor mutant mice. *J Pharmacol Exp Ther* 313(3): 995-1002,2005. PMID: 15764735.
32. **Gerolami A, Marteau C, Matteo A, Sahel J, Portugal H, Pauli AM, et al.** Calcium carbonate saturation in human pancreatic juice: possible role of ductal H+ secretion. *Gastroenterology* 96(3): 881-884,1989. PMID: 2914648.
33. **Gray MA, Greenwell JR, Garton AJ and Argent BE**. Regulation of maxi-K^+ channels on pancreatic duct cells by cyclic AMP-dependent phosphorylation. *J Membr Biol* 115(3): 203-215,1990. PMID: 1695685.
34. **Gray MA, Harris A, Coleman L, Greenwell JR and Argent BE**. Two types of chloride channel on duct cells cultured from human fetal pancreas. *Am J Physiol* 257(2 Pt 1): C240-251,1989. PMID: 2475028.
35. **Gray MA, Winpenny JP, Porteous DJ, Dorin JR and Argent BE**. CFTR and calcium-activated chloride currents in pancreatic duct cells of a transgenic CF mouse. *Am J Physiol* 266(1 Pt 1): C213-221,1994. PMID: 7508188.
36. **Gross E, Fedotoff O, Pushkin A, Abuladze N, Newman D and Kurtz I**. Phosphorylation-induced modulation of pNBC1 function: distinct roles for the amino- and carboxy-termini. *J Physiol* 549(Pt 3): 673-682,2003. PMID: 12730338.
37. **Gross E, Hawkins K, Abuladze N, Pushkin A, Cotton CU, Hopfer U, et al.** The stoichiometry of the electrogenic sodium bicarbonate cotransporter NBC1 is cell-type dependent. *J Physiol* 531(Pt 3): 597-603,2001. PMID: 11251043.
38. **Grundy D, Hutson D and Scratcherd T**. The response of the pancreas of the anaesthetized cat to secretin before, during and after reversible vagal blockade. *J Physiol* 342: 517-526,1983. PMID: 6631748.
39. **Guggino WB**. The cystic fibrosis transmembrane regulator forms macromolecular complexes with PDZ domain scaffold proteins. *Proc Am Thorac Soc* 1(1): 28-32,2004. PMID: 16113408.
40. **Gyr K, Beglinger C, Fried M, Grotzinger U, Kayasseh L, Stalder GA, et al.** Plasma secretin and pancreatic response to various stimulants including a meal. *Am J Physiol* 246(5 Pt 1): G535-542,1984. PMID: 6720952.
41. **Hart WM aTJ**. Bicarbonate and chloride of pancreatic juice secreated in response to various stimuli. *Gastroenterology* 4(409-420),1945. PMID.
42. **Hatefi Y and Hanstein WG**. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. *Proc Natl Acad Sci U S A* 62(4): 1129-1136,1969. PMID: 5256411.
43. **He P, Klein J and Yun CC**. Activation of Na^+/H^+ exchanger NHE3 by angiotensin II is mediated by inositol 1,4,5-triphosphate (IP3) receptor-binding protein released with IP3 (IRBIT) and Ca^{2+}/calmodulin-dependent protein kinase II. *J Biol Chem* 285(36): 27869-27878,2010. PMID: 20584908.
44. **He P, Zhang H and Yun CC**. IRBIT, inositol 1,4,5-triphosphate (IP3) receptor-binding protein released with IP3, binds Na^+/H^+ exchanger NHE3 and activates NHE3 activity in response to calcium. *J Biol Chem* 283(48): 33544-33553,2008. PMID: 18829453.
45. **Henderson LJ**. The Regulation of Neutrality in the Animal Body. *Science* 37(950): 389-395,1913. PMID: 17795147.
46. **Holst JJ, Fahrenkrug J, Knuhtsen S, Jensen SL, Poulsen SS and Nielsen OV**. Vasoactive intestinal polypeptide (VIP) in the pig pancreas: role of VIPergic nerves in control of fluid and bicarbonate secretion. *Regul Pept* 8(3): 245-259,1984. PMID: 6379759.
47. **Hong JH, Muhammad E, Zheng C, Hershkovitz E, Alkrinawi S, Loewenthal N, et al.** Essential role of carbonic anhydrase XII in secretory gland fluid and HCO_3^- secretion revealed by disease causing human mutation. *J Physiol* 593(24): 5299-5312,2015. PMID: 26486891.
48. **Huang CL, Cha SK, Wang HR, Xie J and Cobb MH**. WNKs: protein kinases with a unique kinase domain. *Exp Mol Med* 39(5): 565-573,2007. PMID: 18059132.
49. **Huang CL, Yang SS and Lin SH**. Mechanism of regulation of renal ion transport by WNK kinases. *Curr Opin Nephrol Hypertens* 17(5): 519-525,2008. PMID: 18695394.
50. **Hug MJ, Tamada T and Bridges RJ**. CFTR and bicarbonate secretion by [correction of to] epithelial cells. *News Physiol Sci* 18: 38-42,2003. PMID: 12531931.

51. **Ishiguro H, Naruse S, Kitagawa M, Hayakawa T, Case RM and Steward MC**. Luminal ATP stimulates fluid and HCO_3^- secretion in guinea-pig pancreatic duct. *J Physiol* 519 Pt 2: 551-558,1999. PMID: 10457070.
52. **Ishiguro H, Naruse S, Kitagawa M, Mabuchi T, Kondo T, Hayakawa T, et al.** Chloride transport in microperfused interlobular ducts isolated from guinea-pig pancreas. *J Physiol* 539(Pt 1): 175-189,2002. PMID: 11850511.
53. **Ishiguro H, Naruse S, Steward MC, Kitagawa M, Ko SB, Hayakawa T, et al.** Fluid secretion in interlobular ducts isolated from guinea-pig pancreas. *J Physiol* 511 (Pt 2): 407-422,1998. PMID: 9706019.
54. **Ishiguro H, Steward MC, Lindsay AR and Case RM**. Accumulation of intracellular HCO_3^- by Na^+-HCO_3^- cotransport in interlobular ducts from guinea-pig pancreas. *J Physiol* 495 (Pt 1): 169-178,1996. PMID: 8866360.
55. **Ishiguro H, Steward MC, Naruse S, Ko SB, Goto H, Case RM, et al.** CFTR functions as a bicarbonate channel in pancreatic duct cells. *J Gen Physiol* 133(3): 315-326,2009. PMID: 19204187.
56. **Ishiguro H, Steward MC, Wilson RW and Case RM**. Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. *J Physiol* 495 (Pt 1): 179-191,1996. PMID: 8866361.
57. **Johansen PG, Anderson CM and Hadorn B**. Cystic fibrosis of the pancreas. A generalised disturbance of water and electrolyte movement in exocrine tissues. *Lancet* 1(7540): 455-460,1968. PMID: 4170642.
58. **Jun I, Cheng MH, Sim E, Jung J, Suh BL, Kim Y, et al.** Pore dilatation increases the bicarbonate permeability of CFTR, ANO1 and glycine receptor anion channels. *J Physiol* 594(11): 2929-2955,2016. PMID: 26663196.
59. **Jung J and Lee MG**. Role of calcium signaling in epithelial bicarbonate secretion. *Cell Calcium* 55(6): 376-384,2014. PMID: 24598807.
60. **Jung J, Nam JH, Park HW, Oh U, Yoon JH and Lee MG**. Dynamic modulation of ANO1/TMEM16A HCO_3^- permeability by Ca^{2+}/calmodulin. *Proc Natl Acad Sci U S A* 110(1): 360-365,2013. PMID: 23248295.
61. **Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al.** Identification of the cystic fibrosis gene: genetic analysis. *Science* 245(4922): 1073-1080,1989. PMID: 2570460.
62. **Kiselyov K, Wang X, Shin DM, Zang W and Muallem S**. Calcium signaling complexes in microdomains of polarized secretory cells. *Cell Calcium* 40(5-6): 451-459,2006. PMID: 17034849.
63. **Knauf F, Yang CL, Thomson RB, Mentone SA, Giebisch G and Aronson PS**. Identification of a chloride-formate exchanger expressed on the brush border membrane of renal proximal tubule cells. *Proc Natl Acad Sci U S A* 98(16): 9425-9430,2001. PMID: 11459928.
64. **Ko SB, Mizuno N, Yatabe Y, Yoshikawa T, Ishiguro H, Yamamoto A, et al.** Corticosteroids correct aberrant CFTR localization in the duct and regenerate acinar cells in autoimmune pancreatitis. *Gastroenterology* 138(5): 1988-1996,2010. PMID: 20080093.
65. **Ko SB, Naruse S, Kitagawa M, Ishiguro H, Furuya S, Mizuno N, et al.** Aquaporins in rat pancreatic interlobular ducts. *Am J Physiol Gastrointest Liver Physiol* 282(2): G324-331,2002. PMID: 11804854.
66. **Ko SB, Shcheynikov N, Choi JY, Luo X, Ishibashi K, Thomas PJ, et al.** A molecular mechanism for aberrant CFTR-dependent HCO_3^- transport in cystic fibrosis. *EMBO J* 21(21): 5662-5672,2002. PMID: 12411484.
67. **Ko SB, Zeng W, Dorwart MR, Luo X, Kim KH, Millen L, et al.** Gating of CFTR by the STAS domain of SLC26 transporters. *Nat Cell Biol* 6(4): 343-350,2004. PMID: 15048129.
68. **Kohler H, Nustede R, Barthel M and Schafmayer A**. Exocrine pancreatic function in dogs with denervated pancreas. *Pancreas* 2(6): 676-683,1987. PMID: 3325985.
69. **Konturek SJ, Zabielski R, Konturek JW and Czarnecki J**. Neuroendocrinology of the pancreas; role of brain-gut axis in pancreatic secretion. *Eur J Pharmacol* 481(1): 1-14,2003. PMID: 14637169.
70. **Krishnan D, Liu L, Wiebe SA, Casey JR, Cordat E and Alexander RT**. Carbonic anhydrase II binds to and increases the activity of the epithelial sodium-proton exchanger, NHE3. *Am J Physiol Renal Physiol* 309(4): F383-392,2015. PMID: 26041446.
71. **LaRusch J, Jung J, General IJ, Lewis MD, Park HW, Brand RE, et al.** Mechanisms of CFTR functional variants that impair regulated bicarbonate permeation and increase risk for pancreatitis but not for cystic fibrosis. *PLoS Genet* 10(7): e1004376,2014. PMID: 25033378.
72. **Lee JH, Richter W, Namkung W, Kim KH, Kim E, Conti M, et al.** Dynamic regulation of cystic fibrosis transmembrane conductance regulator by competitive interactions of molecular adaptors. *J Biol Chem* 282(14): 10414-10422,2007. PMID: 17244609.
73. **Lee MG, Ahn W, Choi JY, Luo X, Seo JT, Schultheis PJ, et al.** Na^+-dependent transporters mediate HCO_3^- salvage across the luminal membrane of the main pancreatic duct. *J Clin Invest* 105(11): 1651-1658,2000. PMID: 10841524.
74. **Lee MG aMS**. Physiology of duct cell secretion. *Pancreas: An Integrated Textbook of Basic Science,*

Medicine, and Surgery. BM Beger H, Kozarek R, Lerch M, Neoptolemos J, Warshaw A, Whitcomb D, Shiratori K. . Oxford, U.K, Blackwell Publishing**:** 78-90, 2008.
75. **Lee MG, Ohana E, Park HW, Yang D and Muallem S**. Molecular mechanism of pancreatic and salivary gland fluid and HCO_3 secretion. *Physiol Rev* 92(1): 39-74,2012. PMID: 22298651.
76. **Lee MG, Xu X, Zeng W, Diaz J, Wojcikiewicz RJ, Kuo TH, et al.** Polarized expression of Ca^{2+} channels in pancreatic and salivary gland cells. Correlation with initiation and propagation of [Ca^{2+}]i waves. *J Biol Chem* 272(25): 15765-15770,1997. PMID: 9188472.
77. **Li X, Liu Y, Alvarez BV, Casey JR and Fliegel L**. A novel carbonic anhydrase II binding site regulates NHE1 activity. *Biochemistry* 45(7): 2414-2424,2006. PMID: 16475831.
78. **Lifson N, Kramlinger KG, Mayrand RR and Lender EJ**. Blood flow to the rabbit pancreas with special reference to the islets of Langerhans. *Gastroenterology* 79(3): 466-473,1980. PMID: 7000613.
79. **Linsdell P, Tabcharani JA, Rommens JM, Hou YX, Chang XB, Tsui LC, et al.** Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. *J Gen Physiol* 110(4): 355-364,1997. PMID: 9379168.
80. **Lohi H, Kujala M, Kerkela E, Saarialho-Kere U, Kestila M and Kere J**. Mapping of five new putative anion transporter genes in human and characterization of SLC26A6, a candidate gene for pancreatic anion exchanger. *Genomics* 70(1): 102-112,2000. PMID: 11087667.
81. **Loiselle FB, Morgan PE, Alvarez BV and Casey JR**. Regulation of the human NBC3 Na^+/HCO_3^- cotransporter by carbonic anhydrase II and PKA. *Am J Physiol Cell Physiol* 286(6): C1423-1433,2004. PMID: 14736710.
82. **Luo X, Zheng W, Yan M, Lee MG and Muallem S**. Multiple functional P2X and P2Y receptors in the luminal and basolateral membranes of pancreatic duct cells. *Am J Physiol* 277(2 Pt 1): C205-215,1999. PMID: 10444396.
83. **Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ and Verkman AS**. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem* 274(29): 20071-20074,1999. PMID: 10400615.
84. **Madden ME and Sarras MP, Jr.** Distribution of Na+,K+-ATPase in rat exocrine pancreas as monitored by K+-NPPase cytochemistry and [3H]-ouabain binding: a plasma membrane protein found primarily to be ductal cell associated. *J Histochem Cytochem* 35(12): 1365-1374,1987. PMID: 2824600.
85. **Marteau C, Blanc G, Devaux MA, Portugal H and Gerolami A**. Influence of pancreatic ducts on saturation of juice with calcium carbonate in dogs. *Dig Dis Sci* 38(11): 2090-2097,1993. PMID: 8223086.
86. **Martinez JR**. Ion transport and water movement. *J Dent Res* 66 Spec No: 638-647,1987. PMID: 3305642.
87. **McKenna R and Frost SC**. Overview of the carbonic anhydrase family. *Subcell Biochem* 75: 3-5,2014. PMID: 24146371.
88. **Melvin JE, Yule D, Shuttleworth T and Begenisich T**. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. *Annu Rev Physiol* 67: 445-469,2005. PMID: 15709965.
89. **Min X, Lee BH, Cobb MH and Goldsmith EJ**. Crystal structure of the kinase domain of WNK1, a kinase that causes a hereditary form of hypertension. *Structure* 12(7): 1303-1311,2004. PMID: 15242606.
90. **Muhammad E, Leventhal N, Parvari G, Hanukoglu A, Hanukoglu I, Chalifa-Caspi V, et al.** Autosomal recessive hyponatremia due to isolated salt wasting in sweat associated with a mutation in the active site of Carbonic Anhydrase 12. *Hum Genet* 129(4): 397-405,2011. PMID: 21184099.
91. **Namkung W, Han W, Luo X, Muallem S, Cho KH, Kim KH, et al.** Protease-activated receptor 2 exerts local protection and mediates some systemic complications in acute pancreatitis. *Gastroenterology* 126(7): 1844-1859,2004. PMID: 15188179.
92. **Namkung W, Lee JA, Ahn W, Han W, Kwon SW, Ahn DS, et al.** Ca2+ activates cystic fibrosis transmembrane conductance regulator- and Cl^- -dependent HCO_3 transport in pancreatic duct cells. *J Biol Chem* 278(1): 200-207,2003. PMID: 12409301.
93. **Namkung W, Yoon JS, Kim KH and Lee MG**. PAR2 exerts local protection against acute pancreatitis via modulation of MAP kinase and MAP kinase phosphatase signaling. *Am J Physiol Gastrointest Liver Physiol* 295(5): G886-894,2008. PMID: 18755806.
94. **Nguyen TD, Meichle S, Kim US, Wong T and Moody MW**. P2Y(11), a purinergic receptor acting via cAMP, mediates secretion by pancreatic duct epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 280(5): G795-804,2001. PMID: 11292586.
95. **Nguyen TD, Moody MW, Steinhoff M, Okolo C, Koh DS and Bunnett NW**. Trypsin activates pancreatic duct epithelial cell ion channels through proteinase-activated receptor-2. *J Clin Invest* 103(2): 261-269,1999. PMID: 9916138.
96. **North RA**. Molecular physiology of P2X receptors. *Physiol Rev* 82(4): 1013-1067,2002. PMID: 12270951.

97. **Novak I**. ATP as a signaling molecule: the exocrine focus. *News Physiol Sci* 18: 12-17,2003. PMID: 12531926.
98. **Novak I**. Purinergic receptors in the endocrine and exocrine pancreas. *Purinergic Signal* 4(3): 237-253,2008. PMID: 18368520.
99. **Novak I and Greger R**. Electrophysiological study of transport systems in isolated perfused pancreatic ducts: properties of the basolateral membrane. *Pflugers Arch* 411(1): 58-68,1988. PMID: 3353213.
100. **Ohana E, Yang D, Shcheynikov N and Muallem S**. Diverse transport modes by the solute carrier 26 family of anion transporters. *J Physiol* 587(Pt 10): 2179-2185,2009. PMID: 19015189.
101. **Pallagi P, Hegyi P and Rakonczay Z, Jr.** The Physiology and Pathophysiology of Pancreatic Ductal Secretion: The Background for Clinicians. *Pancreas* 44(8): 1211-1233,2015. PMID: 26465950.
102. **Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, et al.** Dynamic regulation of CFTR bicarbonate permeability by [Cl-]i and its role in pancreatic bicarbonate secretion. *Gastroenterology* 139(2): 620-631,2010. PMID: 20398666.
103. **Park M, Ko SB, Choi JY, Muallem G, Thomas PJ, Pushkin A, et al.** The cystic fibrosis transmembrane conductance regulator interacts with and regulates the activity of the HCO_3^- salvage transporter human Na^+-HCO_3^- cotransport isoform 3. *J Biol Chem* 277(52): 50503-50509,2002. PMID: 12403779.
104. **Park S, Hong JH, Ohana E and Muallem S**. The WNK/SPAK and IRBIT/PP1 pathways in epithelial fluid and electrolyte transport. *Physiology (Bethesda)* 27(5): 291-299,2012. PMID: 23026752.
105. **Park S, Shcheynikov N, Hong JH, Zheng C, Suh SH, Kawaai K, et al.** Irbit mediates synergy between Ca^{2+} and cAMP signaling pathways during epithelial transport in mice. *Gastroenterology* 145(1): 232-241,2013. PMID: 23542070.
106. **Petersen OH**. Stimulus-excitation coupling in plasma membranes of pancreatic acinar cells. *Biochim Biophys Acta* 694(2): 163-184,1982. PMID: 6128029.
107. **Petersen OH and Tepikin AV**. Polarized calcium signaling in exocrine gland cells. *Annu Rev Physiol* 70: 273-299,2008. PMID: 17850212.
108. **Piala AT, Moon TM, Akella R, He H, Cobb MH and Goldsmith EJ**. Chloride sensing by WNK1 involves inhibition of autophosphorylation. *Sci Signal* 7(324): ra41,2014. PMID: 24803536.
109. **Poulsen JH, Fischer H, Illek B and Machen TE**. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci U S A* 91(12): 5340-5344,1994. PMID: 7515498.
110. **Preshaw RM, Cooke AR and Grossman MI**. Quantitative aspects of response of canine pancreas to duodenal acidification. *Am J Physiol* 210(3): 629-634,1966. PMID: 5933217.
111. **Pushkin A, Abuladze N, Lee I, Newman D, Hwang J and Kurtz I**. Cloning, tissue distribution, genomic organization, and functional characterization of NBC3, a new member of the sodium bicarbonate cotransporter family. *J Biol Chem* 274(23): 16569-16575,1999. PMID: 10347222.
112. **Quinton PM**. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. *Lancet* 372(9636): 415-417,2008. PMID: 18675692.
113. **Quinton PM**. The neglected ion: HCO_3^-. *Nat Med* 7(3): 292-293,2001. PMID: 11231624.
114. **Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al.** Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245(4922): 1066-1073,1989. PMID: 2475911.
115. **Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al.** Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245(4922): 1059-1065,1989. PMID: 2772657.
116. **Roos A and Boron WF**. Intracellular pH. *Physiol Rev* 61(2): 296-434,1981. PMID: 7012859.
117. **Roussa E**. Channels and transporters in salivary glands. *Cell Tissue Res* 343(2): 263-287,2011. PMID: 21120532.
118. **Schaffalitzky de Muckadell OB, Fahrenkrug J, Watt-Boolsen S and Worning H**. Pancreatic response and plasma secretin concentration during infusion of low dose secretin in man. *Scand J Gastroenterol* 13(3): 305-311,1978. PMID: 755275.
119. **Schroeder BC, Cheng T, Jan YN and Jan LY**. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* 134(6): 1019-1029,2008. PMID: 18805094.
120. **Shcheynikov N, Kim KH, Kim KM, Dorwart MR, Ko SB, Goto H, et al.** Dynamic control of cystic fibrosis transmembrane conductance regulator $Cl^-/HCO3^-$ selectivity by external Cl^-. *J Biol Chem* 279(21): 21857-21865,2004. PMID: 15010471.
121. **Shcheynikov N, Son A, Hong JH, Yamazaki O, Ohana E, Kurtz I, et al.** Intracellular Cl^- as a signaling ion that potently regulates Na^+/HCO_3^- transporters. *Proc Natl Acad Sci U S A* 112(3): E329-337,2015. PMID: 25561556.

122. **Shcheynikov N, Wang Y, Park M, Ko SB, Dorwart M, Naruse S, et al.** Coupling modes and stoichiometry of Cl^-/HCO_3^- exchange by slc26a3 and slc26a6. *J Gen Physiol* 127(5): 511-524,2006. PMID: 16606687.
123. **Shcheynikov N, Yang D, Wang Y, Zeng W, Karniski LP, So I, et al.** The Slc26a4 transporter functions as an electroneutral $Cl^-/I^-/HCO_3^-$ exchanger: role of Slc26a4 and Slc26a6 in I^- and HCO_3^- secretion and in regulation of CFTR in the parotid duct. *J Physiol* 586(16): 3813-3824,2008. PMID: 18565999.
124. **Shirakabe K, Priori G, Yamada H, Ando H, Horita S, Fujita T, et al.** IRBIT, an inositol 1,4,5-trisphosphate receptor-binding protein, specifically binds to and activates pancreas-type Na^+/HCO_3^- cotransporter 1 (pNBC1). *Proc Natl Acad Sci U S A* 103(25): 9542-9547,2006. PMID: 16769890.
125. **Short DB, Trotter KW, Reczek D, Kreda SM, Bretscher A, Boucher RC, et al.** An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J Biol Chem* 273(31): 19797-19801,1998. PMID: 9677412.
126. **Smith ZD, Caplan MJ, Forbush B, 3rd and Jamieson JD**. Monoclonal antibody localization of Na^+-K^+-ATPase in the exocrine pancreas and parotid of the dog. *Am J Physiol* 253(2 Pt 1): G99-109,1987. PMID: 2441610.
127. **Sohma Y, Gray MA, Imai Y and Argent BE**. HCO3- transport in a mathematical model of the pancreatic ductal epithelium. *J Membr Biol* 176(1): 77-100,2000. PMID: 10882430.
128. **Sohma Y, Gray MA, Imai Y and Argent BE**. A mathematical model of the pancreatic ductal epithelium. *J Membr Biol* 154(1): 53-67,1996. PMID: 8881027.
129. **Steward MC, Ishiguro H and Case RM**. Mechanisms of bicarbonate secretion in the pancreatic duct. *Annu Rev Physiol* 67: 377-409,2005. PMID: 15709963.
130. **Stewart AK, Yamamoto A, Nakakuki M, Kondo T, Alper SL and Ishiguro H**. Functional coupling of apical Cl^-/HCO_3^- exchange with CFTR in stimulated HCO_3^- secretion by guinea pig interlobular pancreatic duct. *Am J Physiol Gastrointest Liver Physiol* 296(6): G1307-1317,2009. PMID: 19342507.
131. **Suzuki J, Umeda M, Sims PJ and Nagata S**. Calcium-dependent phospholipid scrambling by TMEM16F. *Nature* 468(7325): 834-838,2010. PMID: 21107324.
132. **Ulrich CD, 2nd, Holtmann M and Miller LJ**. Secretin and vasoactive intestinal peptide receptors: members of a unique family of G protein-coupled receptors. *Gastroenterology* 114(2): 382-397,1998. PMID: 9453500.
133. **Veel T, Villanger O, Holthe MR, Cragoe EJ, Jr. and Raeder MG**. Na^+-H^+ exchange is not important for pancreatic HCO_3^- secretion in the pig. *Acta Physiol Scand* 144(3): 239-246,1992. PMID: 1316712.
134. **Venglovecz V, Rakonczay Z, Jr., Ozsvari B, Takacs T, Lonovics J, Varro A, et al.** Effects of bile acids on pancreatic ductal bicarbonate secretion in guinea pig. *Gut* 57(8): 1102-1112,2008. PMID: 18303091.
135. **Vilas G, Krishnan D, Loganathan SK, Malhotra D, Liu L, Beggs MR, et al.** Increased water flux induced by an aquaporin-1/carbonic anhydrase II interaction. *Mol Biol Cell* 26(6): 1106-1118,2015. PMID: 25609088.
136. **Wang Y, Soyombo AA, Shcheynikov N, Zeng W, Dorwart M, Marino CR, et al.** Slc26a6 regulates CFTR activity in vivo to determine pancreatic duct HCO_3^- secretion: relevance to cystic fibrosis. *EMBO J* 25(21): 5049-5057,2006. PMID: 17053783.
137. **Willoughby D and Cooper DM**. Organization and Ca^{2+} regulation of adenylyl cyclases in cAMP microdomains. *Physiol Rev* 87(3): 965-1010,2007. PMID: 17615394.
138. **Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, et al.** Human hypertension caused by mutations in WNK kinases. *Science* 293(5532): 1107-1112,2001. PMID: 11498583.
139. **Wizemann V and Schulz I**. Influence of amphotericin, amiloride, ionophores, and 2,4-dinitrophenol on the secretion of the isolated cat's pancreas. *Pflugers Arch* 339(4): 317-338,1973. PMID: 4735613.
140. **Yang D, Li Q, So I, Huang CL, Ando H, Mizutani A, et al.** IRBIT governs epithelial secretion in mice by antagonizing the WNK/SPAK kinase pathway. *J Clin Invest* 121(3): 956-965,2011. PMID: 21317537.
141. **Yang D, Shcheynikov N and Muallem S**. IRBIT: it is everywhere. *Neurochem Res* 36(7): 1166-1174,2011. PMID: 21152975.
142. **Yang D, Shcheynikov N, Zeng W, Ohana E, So I, Ando H, et al.** IRBIT coordinates epithelial fluid and HCO_3^- secretion by stimulating the transporters pNBC1 and CFTR in the murine pancreatic duct. *J Clin Invest* 119(1): 193-202,2009. PMID: 19033647.
143. **Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, et al.** TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* 455(7217): 1210-1215,2008. PMID: 18724360.
144. **You CH, Rominger JM and Chey WY**. Potentiation effect of cholecystokinin-octapeptide on pancreatic bicarbonate secretion stimulated by a physiologic dose of secretin in humans. *Gastroenterology* 85(1): 40-45,1983. PMID: 6303892.
145. **Zeng W, Lee MG and Muallem S**. Membrane-specific regulation of Cl^- channels by purinergic receptors in rat submandibular gland acinar and duct cells. *J Biol Chem* 272(52): 32956-32965,1997. PMID: 9407075.
146. **Zeng W, Lee MG, Yan M, Diaz J, Benjamin I, Marino CR, et al.** Immuno and functional characterization of

CFTR in submandibular and pancreatic acinar and duct cells. *Am J Physiol* 273(2 Pt 1): C442-455,1997. PMID: 9277342.

147. **Zhao H and Muallem S**. Na^+, K^+, and Cl^- transport in resting pancreatic acinar cells. *J Gen Physiol* 106(6): 1225-1242,1995. PMID: 8786358.
148. **Zhao H, Star RA and Muallem S**. Membrane localization of H^+ and HCO_3^- transporters in the rat pancreatic duct. *J Gen Physiol* 104(1): 57-85,1994. PMID: 7964596.