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Molecular Mechanism of Pancreatic and Salivary Glands Fluid and HCO_3^- Secretion

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Abstract

Fluid and HCO_3^- secretion is a vital function of all epithelia and is required for the survival of the tissue. Aberrant fluid and HCO_3^- secretion is associated with many epithelial diseases, such as cystic fibrosis, pancreatitis, Sjögren's syndrome and other epithelial inflammatory and autoimmune diseases. Significant progress has been made over the last 20 years in our understanding of epithelial fluid and HCO_3^- secretion, in particular by secretory glands. Fluid and HCO_3^- secretion by secretory glands is a two step process. Acinar cells secrete isotonic fluid in which the major salt is NaCl. Subsequently, the duct modifies the volume and electrolyte composition of the fluid to absorb the Cl^- and secrete HCO_3^-. The relative volume secreted by acinar and duct cells and modification of electrolyte composition of the secreted fluids varies among secretory glands to meet their physiological functions. In the pancreas, acinar cells secrete small amount of NaCl-rich fluid, while the duct absorbs the Cl^- and secretes HCO_3^- and the bulk of the fluid in the pancreatic juice. Fluid secretion appears to be driven by active HCO_3^- secretion. In the salivary glands, acinar cells secrete the bulk of the fluid in the saliva that contains high concentrations of Na^+ and Cl^- and fluid secretion is mediated by active Cl^- secretion. The salivary glands duct absorbs both the Na^+ and Cl^- and secretes K^+ and HCO_3^-. In this review, we focus on the molecular mechanism of fluid and HCO_3^- secretion by the pancreas and salivary glands, to highlight the similarities of the fundamental mechanisms of acinar and duct cell functions, and point the differences to meet glands specific secretions.

I. INTRODUCTION

Bicarbonate (HCO_3^-) is an indispensible ion in secreted fluids, including the pancreatic juice and saliva. Among other functions, HCO_3^- is the biological pH buffer that guards against toxic intracellular and extracellular fluctuations in pH (365). As a chaotropic ion, HCO_3^- facilitates solubilization of macromolecules (like digestive enzymes and mucins) in biological fluids and stimulates mucin secretion (45, 145, 410). HCO_3^- secreted by the exocrine pancreas neutralizes gastric acid and provides an optimal pH environment for digestive enzymes function in the duodenum (237). HCO_3^- secretion into the oral cavity protects against enamel erosion by acidic pH (192). Indeed, recent progress in epithelial biology indicates that aberrant HCO_3^- transport has a fundamental role in human

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pathophysiology (346, 347). For example, in cystic fibrosis (CF) abnormal HCO_3^- secretion leads to altered mucin hydration and solubilization (348), resulting in thick mucus that frequently blocks ductal structures of the internal organs. Therefore, altered HCO_3^- secretion is associated with a wide spectrum of diseases and disorders of epithelial tissues including respiratory, gastrointestinal, and genitourinary systems (61, 284, 346, 347, 432).

At pH 7.4 and 5% CO_2, the HCO_3^- equilibrium concentration is approximately 25 mM. Several bodily fluids have higher HCO_3^- concentration, and among them the pancreatic juice contains the highest concentration. In humans and several other species, such as dogs, cats, and guinea pigs, HCO_3^- concentration in the juice secreted by the stimulated pancreas can be higher than 140 mM (86, 237). This remarkable transport feat attracts considerable attention to pancreatic HCO_3^- secretory mechanism, which is the model of choice to gain insight into the mechanism of epithelial fluid and HCO_3^- transport. How exocrine glands secrete copious amount of fluid and HCO_3^- has long been a puzzle. The discovery of acidic pancreatic juice in patients with CF was a milestone in understanding the physiological mechanisms of pancreatic HCO_3^- secretion (191). In addition, significant progress has been made during the last 20 years with the identification of the molecular nature of many exocrine glands ion channels and transporters, including the cystic fibrosis transmembrane conductance regulator (CFTR) (199), the Na^+-HCO_3^- co-transporter NBCe1-B (also known an pNBC1) (1) and the SLC26 transporters (91, 314). Regulation and coordination of exocrine HCO_3^- secretion is being defined with understanding the role of regulatory proteins, such as PSD95/discs large/ZO-1 (PDZ)-based adaptor proteins, with-no-lysine (WNK) kinases, the SPAK/OSR1 kinases and of the inositol-1,4,5-triphosphate (IP_3) receptor binding protein released with IP_3 (IRBIT). However, we have just begun to uncover how the transporting proteins are organized into complexes that function in concert in the luminal (apical) and basolateral membranes and how the high concentration of HCO_3^- in formed and maintained in the luminal space of exocrine glands.

Another cardinal aspect of exocrine gland function is fluid secretion. While HCO_3^- secretion is mostly carried out by the gland ducts, the bulk of fluid secretion can be by the duct, as in the exocrine pancreas (237, 404), or by acinar cells, as in the salivary glands (274, 368). While the ionic bases of fluid secretion by the duct are poorly understood, the fundamental mechanism of acinar cell fluid secretion is fairly well characterized. Early mechanistic work defined the basic transport mechanisms at the BLM and LM (331). More recent work relied on gene deletion in mice (50, 369), which confirmed the basic mechanism, but also resulted in unexpected surprises as to the diversity and function of transporter isoforms.

This review is aimed at consolidating the current knowledge of exocrine glands fluid and HCO_3^- secretion. Since it is understood best, fluid secretion by salivary glands will be emphasized as an example for the mechanism of fluid secretion by acinar cells. Ductal function will be discussed in relation to the molecular mechanism of pancreatic HCO_3^- secretion with an attempt to explain how the pancreatic juice can accumulate 140 mM HCO_3^-, a concentration that is more than five times higher than that found in plasma. We will also briefly discuss fluid and HCO_3^- transport by salivary gland ducts to demonstrate adaptation and alteration of the basic mechanism to meat tissue specific demands.

II. GENERAL CONSIDERATIONS

A. Overview

When Bayliss and Starling described the discovery of the pancreatic secretagogue secretin, they also noticed that the exocrine pancreas secretes alkaline fluids (25). At the time, they assumed that carbonate is responsible for the strong alkalinity of the pancreatic juice. Later,

with better understanding of the carbonate/HCO_3^-/CO_2 buffer systems (156), 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 (46, 87, 143). Exocrine glands secrete macromolecules like digestive enzymes and mucins immersed in a HCO_3^--rich fluid. Exocrine glands secretion is a two step process (412). The digestive enzymes are synthesized and secreted by the acinar cells. Depending on the gland, acinar cells also secrete a small (pancreas) or a large (salivary) volume of isotonic, plasma-like, NaCl-rich fluid (237, 274). The fluid secreted by acinar cells deliver the macromolecules to the duct. The duct modifies the ionic composition of the fluid along the ductal tree to absorb most of the Cl^- and secrete the bulk of the HCO_3^-. The pancreatic duct also secretes most of the fluid in the pancreatic juice (14, 404) and the salivary duct absorbs the Na^+ and secret K^+ to form the final saliva (368). Hence, the ductal tree has several functions, providing a structural framework for acinar and endocrine tissues, secreting fluid that acts as a vehicle for the transport of macromolecules out of the glands, and secreting HCO_3^- to neutralize acid and provide optimal pH environment for the secreted macromolecules at their destination (14, 44).

B. Morphology

Below, we describe the anatomy of the pancreas as an example of an exocrine gland. However, the anatomy of other exocrine glands (like the salivary, lacrimal and mammary) is similarly organized. The pancreas is a complex endocrine-exocrine organ, with each part developed from the ventral and dorsal surfaces of the primitive foregut, respectively. Similar to other exocrine glands, the exocrine pancreas is composed of two major cell types, the acinar and duct cells. In humans and most other mammals, acinar cells comprise the major mass of the pancreas and duct cells comprise only about 10% of the cells in number and 5% of the total pancreatic gland weight (44). The terminal portion of the ductal tree leading directly from the acinus is called intercalated duct (Fig. 1).

The acinar cells are the classical model for polarized epithelial cells, having a typical morphology with extensive rough ER at the basal pole and smooth ER at the apical pole. The nucleus is close to the basal pole and is followed by the Golgi apparatus, the secretory granules that are packed at the apical pole, and the luminal membrane (271). The polarity is extended to other intracellular organelles and to single proteins. A good example is the Ca^{2+} signaling proteins. These proteins are organized as Ca^{2+} signaling complexes next or at the tight junctions, with polarized expression of Ca^{2+} pumps (239, 474), Ca^{2+} channels (240), G protein-coupled receptors (392) and regulatory proteins (163), that are essential for generating the localized Ca^{2+} signal and the propagated Ca^{2+} waves (208, 337).

The acinar lobule terminates and the duct starts with the centroacinar cells, which have several ductal characteristics, are regarded as the terminal cells of the ductal tree and connect the acinar and duct cells (237). The duct has several segments based on size and location. Although here we emphasize the secretory (serous) duct cells, it is important to note that the duct has several cell types, including mucosal and ciliated cells. The cellular heterogeneity is found in all segments of the ductal tree. Small intercalated ducts join together and sequentially form the intralobular, interlobular, and interlobar duct segments (Fig. 1). In humans, the interlobar ducts join to form the main pancreatic duct (duct of Wirsung), which shares a duodenal opening with the common bile duct at the Ampulla of Vater. Most individuals have one main pancreatic duct, but some have an additional accessory pancreatic duct, the Duct of Santorini (34). In rodents, a number of interlobular or interlobar ducts open directly into the common pancreaticobiliary duct without forming a main duct (237). Much attention is devoted in recent years to developmental aspects of the duct, since the duct contains the glandular stem cells that are necessary for development and gland repair after injury (195, 219, 248).

The intercalated and small intralobular ducts are the major sites of HCO_3^- secretion in the human pancreas and salivary glands, whereas in rodents the interlobular duct secretes the bulk of the fluid and HCO_3^- (259). The HCO_3^--secreting portion of these ducts is lined by the principal cells. These cells contain a relatively small amount of cellular organelles required for protein secretion, such as rough endoplasmic reticulum (RER), Golgi complexes and secretory vesicles. Instead, they are rich with mitochondria to satisfy the energy demand of transcellular HCO_3^- and fluid secretion. The luminal membrane of the principal cells is endowed with extensive microvilli. The lateral membrane is interdigitated and linked by tight and adherent junctions and desmosomes. In the larger ducts the principal cells become columnar and the duct contains goblet cells, which are specialized in mucin secretion (44, 257).

C. Electrolyte Composition of the Secreted Fluids

The human pancreas secretes 1–2 L of pancreatic juice per day. The pancreatic juice is a alkaline, isotonic fluid. The acini secrete isotonic, plasma-like fluid. The pancreatic duct does not absorb the Na^+, but absorbs most of the Cl^- and secret HCO_3^-. The human pancreatic duct can secrete a fluid containing 140 mM HCO_3^- (86). However, the HCO_3^- concentration in mouse and rat pancreatic juice is 50–70 mM (386). The HCO_3^- content in the juice increases with increased flow rate. The peak HCO_3^- content is reached at 30–50% of maximal flow rate. The reciprocal Cl^- absorption and HCO_3^- secretion results in isotonic osmolality at all flow rates (46, 358, 386). The cation composition of the juice is nearly constant, 140 mM Na^+ and 10–15 mM K^+, regardless of flow rate. Human juice also contains 1–2 mM Ca^{2+} and a small amount of Mg^{2+}, Zn^{2+}, PO_4^{3-}, and SO_4^{2-}.

Humans secrete about 1 L saliva per day. The resting secretion is dominated by the submandibular and sublingual glands, whereas the stimulated secretion is mainly by the parotid gland (369). As is the case in the pancreas, electrolyte composition of the saliva varies with flow rate and among species. Unlike the pancreas, most salivary fluid is secreted by the serous acinar cells, which secrete isotonic, NaCl-rich fluid. The primary fluid in the rat and cat contains 145–160 mM Na^+, 5–10 K^+, 120–130 Cl^- and pH of about 7.5 (382). The salivary gland ducts express the epithelial Na^+ channel ENaC (65) and absorbs most of the Na^+ and Cl^- from the primary saliva while secreting K^+ and HCO_3^- to generate the final saliva. The cholinergic stimulated and Ca^{2+}-dependent saliva in the rat and mouse at steady state contains 2 mM Na^+, 135 mM K^+, 55 mM HCO^-_3 and 78 mM Cl^- at a transepithelial potential of −11 mV (66, 462). Interestingly, the β-adrenergic stimulated small volume of saliva contains as much as 140 mM HCO_3^- and as little as 20 mM Cl^- (382, 462). This indicates that cholinergic and β-adrenergic stimulation use different pathways to stimulate fluid and electrolyte secretion.

III. HORMONAL CONTROL OF FLUID AND HCO_3^- SECRETION

A. Overview

Like any physiological system, the function of exocrine glands is controlled by multiple neurohomoral inputs that form intricate regulatory pathways. Here we focus our discussion on the exocrine pancreas, although for the most part similar mechanisms operate in the salivary glands. The fundamental similarities between the two tissues are that fluid secretion by acinar cells is regulated by Ca^{2+}-mobilizing agonist (274, 334, 337) and fluid and HCO_3^- secretion by duct cells is largely regulated by cAMP generating receptors (237, 265). The most obvious difference that is outside the topic of this review is in enzyme secretion that is Ca^{2+}-activated in the pancreas, but cAMP-activated in the salivary glands. In addition, sympathetic inputs are central in the function of salivary glands, but do not play a role in the function of the exocrine pancreas.

Neurohumoral control of pancreatic fluid and electrolyte secretion was first proposed in the early 20th century (25, 328). Knowledge accumulated over the past 100 years has revealed that regulation of pancreatic secretion is highly complex with multiple stimulatory and inhibitory inputs (398) and varies greatly among species. Basal secretion in rat and mouse is significant, while basal secretion in dog and possibly human is very small, amounting to less than 2% of the HCO_3^- and 10% of the digestive enzymes secreted during maximal stimulation (73). HCO_3^- salvage mechanisms in the large pancreatic duct contribute to the low basal HCO_3^- output (234, 250). Pancreatic enzyme and HCO_3^- secretion increases in response to a meal. Control of pancreatic secretion is divided into cephalic, gastric, and intestinal phases (44). The intestinal phase is the most important and commences with the passage of chyme into the proximal duodenum. The pancreatic acini and duct express receptors for a battery of hormones and neurotransmitters. The major receptors in acini are for the hormones cholecystokinin (CCK) and bombesin and the neurotransmitter acetylcholine. The hormone secretin, secreted by cells in the upper duodenum, and cholinergic vagal output via an enteropancreatic vagovagal reflex are the two principal receptors controlling ductal fluid and HCO_3^- secretion. Secretin and cholecystokinin (CCK) are released by distant organs and require high affinity receptors because they reach the pancreas via the bloodstream (237). In addition to these classical hormones, a large number of humoral agents are released by the pancreas to modulate its function. Cells in the islets of Langerhans release insulin, somatostatin, and several other peptide hormones (243), and paracrine agonists released from pancreatic acinar and duct cells, such as purines, prostaglandins and activated trypsin, regulate duct cell function in physiological and pathological states.

B. Endocrine and Paracrine Control

Cholecystokinin (CCK)—Circulating CCK released from the small intestines is a major stimulator of acinar cells enzyme and fluid secretion. This has been amply demonstrated in several rodent species. CCK acting through an increase in cytoplasmic Ca^{2+} prominently stimulates Ca^{2+}-dependent exocytosis. However, the relevance of CCK-mediated action in human acinar cells has been questioned by several studies, suggested that the effect of CCK is mostly mediated by stimulation of the intestinal vagal afferent fibers (319). This was based on the claim that the human pancreas does not express CCK-A receptors (187, 188, 278). However, this problem was resolved recently with the demonstration of CCK-evoked Ca^{2+} signals and exocytosis in isolated human acinar cells (286). The endogenously released CCK is heterogeneous and consists of multiple forms including CCK-58, CCK-33, CCK-8 (101). Although the exact role of each isoform is not known and in isolated acini they have similar activity (69), there is evidence for differences in their function *in vivo* (453).

CCK also acts on the pancreatic duct to affect fluid secretions, but its effect varies among species. In humans, the infusion of CCK alone weakly stimulates fluid secretion, but it greatly potentiates the effects of secretin (460). This suggests prominent synergism between the Ca^{2+} and cAMP systems in stimulated ductal secretion. This is discussed in more detail in another section of this review.

Secretin—The entry of acidic chyme into the duodenum evokes the release of secretin from neuroendocrine cells in the duodenal mucosa. Intraduodenal pH below 4.5 is a prime stimulus for secretin release (37, 58). Secretin acts mainly on the duct by increasing cAMP to stimulate ductal fluid and HCO_3^- secretion. That secretin is the principal hormone in postprandial fluid and HCO_3^- secretion is evident from 1) a rise in plasma secretin after a meal (58, 341), 2) a linear relationship between the rise in plasma secretin and HCO_3^- output (380) and 3) inhibition of postprandial pancreatic HCO_3^- secretion by serum anti-secretin (57). Plasma secretin in response to a meal reaches only pM levels, which is

sufficient to stimulate modest fluid and HCO_3^- secretion in all species. CCK and vagal stimulation further potentiates the secretin-stimulated secretion (134, 216, 460).

Purines—Purinergic receptors (P2Rs) are classified into metabotropic P2Y and ionotropic P2X receptors and transduce their signal by increasing cytoplasmic Ca^{2+}. Pancreatic acini store and secret ATP but do not appear to express P2Rs (304). ATP released by acini acts on the duct, which expresses multiple P2YRs and P2XRs both at the apical and basolateral membranes (252). Stimulation of P2Rs in the isolated pancreatic ducts from several species induces fluid secretion and activates membrane transporters that enhance HCO_3^- secretion (291, 304, 476). However, P2Rs regulation can be quite complicated, for example stimulations of the apical and basolateral P2Rs have opposite effect on guinea pig ductal secretion (176). Possible sources of purinergic ligands include release by nerve terminals at the basolateral space, release of purines stored in zymogene granules of acinar cells, and efflux by ductal ATP transporters (302, 304). Although the purinergic system likely plays a role in the regulation of pancreatic ductal secretion under physiological and pathological states, its specific role in humans yet to be demonstrated.

Other humoral mediators secreted by islets, the gastrointestinal tract and the intrapancreatic nervous system can also modulate the function of the exocrine pancreas, although their exact physiological function remained to be elucidated (see (237) for further details).

C. Neuronal Control

Pancreatic secretion is controlled by the enteric nervous system, which is comprised of a gut-brain axis and an intrapancreatic system. The intrapancreatic nervous system comprises of interconnecting plexus of ganglia and postganglionic fibers lying in the intralobular connective tissues, blood vessels, and occasionally in the neuronal trunk (207, 217). It is supplied by preganglionic parasympathetic (vagal) fibers, postganglionic sympathetic (splanchic) fibers, and possibly other fibers that emanate directly from the gut wall. Nerve fibers travel through the lamina propria of the acini and the duct, with nerve terminals located in close proximity to the basal membrane without forming synapses (257, 418).

The major neurotransmitter acting on acinar and duct cells is acetylcholine secreted by vagal parasympathetic fibers. The acini and duct express M1 and M3 cholinergic receptors, although the M3 is the main receptor type (113) and acts by increasing cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) (208). Parasympathetic nerve terminals contain additional neurotransmitters, including vasoactive intestinal peptide (VIP) and ATP (217, 303). The effects of vagal stimulation on pancreatic fluid secretion show species-specific pattern and are quite variable. Vagal stimulation in pigs and guinea pigs causes VIP-stimulated fluid and HCO_3^- secretion (162, 217). In humans, cholinergic stimulation results in enzyme secretion by acinar cells and enhances the secretin-stimulated ductal secretion, likely by the Ca^{2+}-cAMP synergy. The synergism between the Ca^{2+} and cAMP system (see below) can explain the strong stimulatory effect of VIP in the pig. VIP (and the β-adrenergic receptors) can stimulate both limbs of the signaling pathways by a Gs/Gi switching mechanism to increase both cellular cAMP and Ca^{2+} (251).

Other neurotransmitters acting on the exocrine pancreas are Neuropeptide Y (NPY), galanin (93, 390) and histidine isoluecine (161) that have modulatory effect, with NPY mainly controlling blood flow to induce vasoconstriction and inhibit pancreatic secretion. Substance P and the calcitonin gene-related peptide (CGRP) are co-localized in the same neuron and act as inhibitory neurotransmitters (56).

Regulation of salivary glands function is not understood to the same extent as pancreatic function. Salivary gland function is also controlled by multiple inputs, endocrine, paracrine

and neuronal (274, 342, 377). The main neuronal regulation is by parasympathetic and sympathetic nerve endings. The parasympathetic neurons release acetylcholine to activate the M1 and M3 receptors in both acinar and duct cells (38, 71, 114, 451) to increase $[Ca^{2+}]_i$ and mainly stimulate fluid secretion. The sympathetic nervous system activates β-adrenergic receptors in acinar and duct cells to increase cAMP (23, 48, 75). β-adrenergic stimulation is the main pathway for stimulated enzyme secretion in acinar cell (48). In the duct, β-adrenergic stimulation controls fluid and electrolyte transport (66), including HCO_3^- secretion (389, 472).

Salivary glands acinar and duct cells express a battery of ionotropic and metabotropic P2 receptors (P2Rs) both in the luminal and basal membranes (415, 465). The P2Y receptors appear most important in gland development (22, 416) and perhaps repair (350). The P2X receptors (287, 307, 465) may have a role in fluid and electrolyte secretion. A recent convincing study showed a prominent stimulation of salivary glands secretion by the P2X7 receptors (287), which are expressed in both acinar and duct cells (242, 354) and act by increasing $[Ca^{2+}]_i$ (242, 287, 307). How the function of all inputs is orchestrated to produce the final saliva (and for that matter, the pancreatic juice) is not known at present.

D. Signaling Pathway in Secretory Glands

Acinar and duct cell functions in both the pancreatic and salivary glands is regulated by receptors that change the free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) (208, 337). Changes in $[Ca^{2+}]_i$ are also critically involved in pancreatic (205, 330) and salivary glands (205) pathology. Ca^{2+} signaling entails receptor-mediated activation of the G proteins Gq to generate Gαq·GTP or Gi to release Gβγ, which activate phospholipase C β (PLCβ). PLCβ hydrolyses PIP2 to generate IP_3 and diacylglycerol (32). IP_3 activates the ER-located Ca^{2+} release channels IP_3 receptors (IP_3Rs) (276), with IP_3R2 and IP_3R3 being the major isoforms in secretory cells (110). Ca^{2+} release is followed by activation of plasma membrane Ca^{2+} influx channels, the so called store-operated channels (SOCs). The two SOCs are the Orai (104, 427, 468) and TRPC channels (167, 447, 463). The TRPC and Orai channels are activated by the ER Ca^{2+} sensor STIM1 (246, 366). In response to Ca^{2+} stores depletion, STIM1 clusters with TRPC (6, 323, 467) and the Orai channels (83, 246, 249, 419) to activate them. The increase in $[Ca^{2+}]_i$ activates the Ca^{2+} extrusion mechanisms, the sarco/endoplasmic Ca^{2+} ATPase (SERCA) pumps and the plasma membrane Ca^{2+} ATPase (PMCA) pumps, to move Ca^{2+} from the cytosol and restore $[Ca^{2+}]_i$ towards the basal level.

The Ca^{2+} signal in epithelial cells is highly polarized, initiating at the apical pole and propagating to the basal pole (196, 414). Polarization of the Ca^{2+} signal is achieved by polarized arrangement of all Ca^{2+} signaling proteins and their assembly into complexes in close proximity of the tight junction. Polarized expression has been demonstrated for all three IP_3Rs (240, 293, 446), the SERCA and PMCA pumps (239, 474), GPCRs (357, 392), TRPC channels (203), Orai channels and STIM1 (163, 253). Such an arrangement launches the Ca^{2+} signal at the cellular domain that initiates the polarized cellular function of the cells, being exocytosis of secretory granules or stimulated fluid and electrolyte secretion.

The Ca^{2+} signal evoked by physiological agonist concentrations is in the form of Ca^{2+} oscillations, where the Ca^{2+} signal is periodically repeated. The frequency and amplitude of the oscillation is determined by the intensity of receptor stimulation (31, 208). The Ca^{2+} oscillations always start at the apical pole and propagate to the basal pole (196, 414) in the form of Ca^{2+} waves (197). In pancreatic acinar cells the oscillations can remain confined to the apical pole (333, 337, 414). Interestingly, the spatial and temporal aspects of the Ca^{2+} oscillations vary among pancreatic and parotid acinar cells (120), likely reflecting adaptation of the Ca^{2+} signal to the specialized function of the two cell types. The Ca^{2+} signaling pathway mediates exocytotic enzyme secretion by pancreatic acini (438, 439), fluid

secretion by pancreatic and salivary glands acini (274, 331), and modulate ductal fluid and electrolyte secretion (see below).

The second signaling pathway is the cAMP/PKA pathway that is activated by receptors coupled to Gs, as is the case in the ducts. The main stimulator of pancreatic ductal secretion is secretin and of salivary ducts function is norepinephrine acting on the β-adrenergic receptors. Secretin receptors belong to the class B G-protein-coupled receptor (GPCR) and β-adrenergic receptors are class A GPCR. Stimulation of the two receptor types in duct cells evokes an increase in cAMP and activation of PKA (66, 75, 88, 100, 417). Stimulation of the VIP receptors in duct cells ($VPAC_1$), also activates the cAMP/PKA pathway (100, 417). The main transporters activated by cAMP to evoke fluid and HCO_3^- secretion are the luminal CFTR and the basolateral Na^+-HCO_3^- cotransporter (237, 369) NBCe1-B (458). In the pancreatic duct, anti-secretin antibodies block about 80% of postprandial HCO_3^- output (57), highlighting the central role of secretin in stimulating HCO_3^- secretion.

In recent years, a more complicated picture of the regulation of ductal fluid and electrolyte secretion has emerged with the realization that pancreatic and salivary gland cells express a multitude of receptor types. In the pancreatic duct, these include multiple P2 receptors (252, 297) and the protease-activated receptor 2 (PAR2) (9, 290, 292, 299), which in addition to stimulating secretion (287), may mediate critical steps in apoptosis (P2X7 receptors) and the inflammatory response (PAR2) associated with pancreatitis. The P2X7 receptors may have different roles in acinar and duct cells since the receptors show cell specific behavior (242), and pore expansion (241). Functional studies suggest expression of P2Y2R and P2X7R at the luminal membrane and perhaps P2Y1R, P2Y2R and P2X4R at the basolateral membrane of the duct (252). Both the P2Y and P2X receptors signal through changes in $[Ca^{2+}]_i$ (302). Subsequently, it was reported that the basolateral P2Rs inhibit HCO_3^- and fluid secretion in guinea pig duct (176). The P2Y11R that signal through changes in cAMP stimulates a luminal Cl^- channel, most likely CFTR (297). The basolateral PAR2 that signals via changes in $[Ca^{2+}]_i$ activates the luminal Ca^{2+}-activated Cl^- and K^+ channels (299) and stimulates ductal HCO_3^- secretion (9). Again, to what extent these various receptors contribute to fluid and HCO_3^- secretion *in vivo* is not clear at present. However, the PAR2 and P2X7 receptors may become particularly active in pathological states when significant trypsin and ATP are released to protect the cells.

E. Synergism

The cAMP and Ca^{2+} signal pathways show prominent synergism in many physiological functions. A particularly well defined synergism is fluid and HCO_3^- secretion by the pancreatic duct and will be used here to illustrate the phenomenon. Although secretin is the primary stimulator of pancreatic fluid and HCO_3^- secretion, exogenous application of secretin that elevates plasma concentration to the level observed in the postprandial state evokes only modest HCO_3^- and fluid output (87, 136). This suggests that other factors, such as CCK and vagal stimulation, synergize with secretin to stimulate ductal fluid and HCO_3^- secretion. A decrease in secretin-evoked HCO_3^- secretion by the cholinergic muscarinic receptors antagonist atropine and by vagal blockade indicates that a vagal cholinergic input is important for postprandial HCO_3^- secretion (134, 216). In addition, exogenous *in vivo* application of CCK potentiates the secretin-stimulated fluid and HCO_3^- secretion (460). Stimulation of both the M1 and M3 and CCK receptors (CCK_A) in pancreatic duct cells evoke an increase in $[Ca^{2+}]_i$ and potentiate the effect of cAMP agonists such as secretin and VIP (237).

The mechanism of synergism between cAMP and Ca^{2+} signals in pancreatic duct cells is not yet fully understood. Activation of Ca^{2+}-activated K^+ channels in the basolateral membrane, which facilitates anion secretion through CFTR in the apical membrane, seems to be one

plausible mechanism that has been demonstrated in several epithelia (60, 226). A second mechanism is Ca^{2+}-dependent activation of CFTR-dependent Cl^-/HCO_3^- exchange shown in CAPAN-1 human pancreatic duct cells (291). This mechanism may involve activation of CFTR by IRBIT. The regulatory role of IRBIT is discussed in detail below and in a recent review (457). For the current discussion, it is necessary to know that IRBIT binds to the IP_3Rs to inhibit their function, and it is released from the IP_3Rs by IP_3 (11). IRBIT also activates both the Na^+-HCO_3^- cotransporter NBCe1-B (394, 458) and CFTR (458). Stimulation of a Ca^{2+} mobilizing receptors that results in an increase in IP_3 may result in dissociation of IRBIT from the IP_3Rs and its binding to NBCe1-B and/or CFTR to activate them and increase the cAMP-activated fluid and HCO_3^- secretion. A reciprocal form of synergism is an augmentation of the $[Ca^{2+}]_i$ signal by cAMP. In parotid acinar cells, cAMP/PKA phosphorylates the IP_3R2 to increase channel open probability and hence augments the Ca^{2+} signal (38). Finally, Ca^{2+} can positively control the cAMP/PKA system. Several adenylyl cyclases (ACs), such as AC1 and AC8, are activated by elevated $[Ca^{2+}]_i$ (440).

VI. FLUID AND ELECTROLYTE SECRETION BY ACINAR CELLS

A. Overview

Fluid and electrolyte secretion by secretory glands is a two step process, in which acinar cells secrete isotonic, plasma-like fluid and the duct modifies the electrolyte composition to generate the final fluid. The volume secreted by acinar cells is different in each gland. For example, pancreatic acinar cells secrete a small amount of volume and most fluid in the final pancreatic juice is secreted by the duct (237, 404). On the other hand, in all salivary glands the serous acinar cells specialize in fluid secretion and secrete most of the fluid in the saliva (274). Yet, to the extent that they are known, the fundamental mechanism of fluid and electrolyte secretion by the two acinar cell types is similar, if not identical. The only two known features that may explain the different rate and volume secreted by pancreatic and salivary gland cells are the properties of their receptor-evoked Ca^{2+} signal, which is much faster in salivary glands acinar cell (120), and perhaps expression of high level of the water channel aquaporin-5 in salivary gland acinar cell (80). Most mechanistical information on pancreatic acinar cells fluid and electrolyte secretion comes from relatively early functional studies and the molecular identity of most transporters is known only to a limited extent (for review see (331)). Most recent work has been done with salivary gland acinar cells and relied on gene deletion in mice, which revealed several unexpected features and role of the transporters (50, 274, 369). Therefore, for the most part, we will discuss fluid and electrolyte secretion by salivary gland cells and will provide information on pancreatic acinar cells when available.

B. Acinar Cells Electrolyte Transporters

The Na^+/K^+ ATPase pump—Fluid and electrolyte secretion is fueled by the cellular Na^+ gradient generated by the basolateral Na^+/K^+ ATPase pump, which hydrolyses ATP to exchange $3Na^+_{in}$ for $2K^+_{out}$ and one H^+_{in} (280) and generate the transcellular Na^+ and K^+ gradients, and thus also determines the membrane potential (331).

K^+ channels—The acinar cell membrane potential is close to the K^+ diffusion potential, which is set by K^+ channels. The membrane potential provides the driving force for the exit of Cl^- at the luminal membrane, which is the key step initiating fluid and electrolyte secretion. Early microelectrodes work recorded K^+ conductance in acinar cells that was activated by Ca^{2+} (332). With the invention of patch-clamp technique (139), it became clear that acinar cells express two type of Ca^{2+}-activated K^+ channels (331), a Ca^{2+}- and voltage-activated K^+ channel of a large conductance (267) and a time- and voltage-independent K^+ channel of intermediate conductance (147, 294). The molecular identity of the channels was

subsequently determined as the MaxiK channels coded by the *Kcnma1* (294, 360) and the mIK1 channels coded by the *Kcnn4* gene (27, 146), respectively. Gene deletion in mice revealed that both channels are required to sustain acinar cells and salivary glands function. Thus, deletion of *Kcnn4* had no effect on either resting or stimulated acinar cell volume regulation or glandular fluid and electrolyte secretion, including K^+ secretion (27). Moreover, deletion of the *Kcnma1* gene had no effect on salivary glands fluid and electrolyte secretion (360), although K^+ secretion is impaired in salivary glands lacking the MaxiK channel that is expressed in the luminal membrane of the duct (288). It was necessary to delete both the *Kcnn4* and the *Kcnma1* genes in mice to reduce receptor-stimulated fluid and K^+ secretion by acinar cells and thus by salivary glands (362). Acinar cells appear quite plastic and lack of effect due to deletion of one of the K^+ channels may result from adaptation of the acinar cells. Example for acinar cells plasticity and adaptability are also seen in deletion of the Na^+/H^+ exchange NHE1, which resulted in extensive adaptation to increased Cl^-/HCO_3^- exchange activity, probably by enhanced expression of carbonic anhydrase II, and increased expression of of NKCC1 (121). Another example is the partial deletion of the ER Ca^{2+} pump SERCA2 that resulted in adaptation of the Ca^{2+} signaling and granule exocytotic machineries (474).

NKCC1—A key transporter for acinar cells fluid and electrolyte secretion is the $Na^+/K^+/2Cl^-$ co-transporter NKCC1. NKCC1 is ubiquitous and is activated by cell shrinkage to mediate regulatory volume increase to restore cell volume after cell shrinkage (78, 225). NKCC1 is expressed in the basolateral membrane of acinar cells (99) and is inhibited by the diuretics furosemide and bumetanide (137). Manipulation of external ions and the use of NKCC inhibitors showed that NKCC activity mediates about 70% of the Cl^- uptake that is secreted across the luminal membrane to drive secretory glands fluid secretion (274, 310, 331). NKCC1 together with the Na^+/H^+ exchanger NHE1 also provide the cytosol with most of the Na^+ necessary to activate the Na^+/K^+ pump (469, 470). The central role of NKCC1 in salivary glands fluid and electrolyte secretion was further established by deletion of the *Nkcc1* gene in mice, which resulted in about 70% inhibition of salivary secretion (99).

NHE1 and AE2—The findings that inhibition (310) and deletion of Nkcc1 in mice (99) inhibited fluid and electrolyte secretion by only 70% indicate that another mechanism can fuel the secretory process. Partial inhibition of glandular secretion by the Na^+/H^+ exchanger inhibitor amiloride and by the Cl^-/HCO_3^- exchanger inhibitor DIDS (227, 266, 310) suggested that these transporters acting in concert mediate part of the basolateral membrane Cl^- uptake necessary for luminal Cl^- secretion. The Na^+/H^+ exchangers family includes five members that are expressed in the plasma membrane NHE1-NHE5 (33, 317) and the Cl^-/HCO_3^- exchangers family includes four members AE1-AE4 (8). Functional (272, 283, 469, 470), immunological (98, 272, 372) and molecular studies (98, 403) identified the acinar cells basolateral membrane Na^+/H^+ exchanger isoform as NHE1 and the Cl^-/HCO_3^- exchanger isoform as AE2. NHE1 and AE2 are ubiquitous, function as the main regulators of cytoplasmic pH, and are activated by small changes in pH_i. NHE1 is activated by acidic pH_i (16) to extrude acid generated by cellular metabolism, while AE2 is activated by alkaline pH_i to extrude excessive cytosolic base (316). By virtue of regulating pH_i in resting and stimulated cells, NHE1 and AE2 are involved in many cellular functions that in secretory gland cells include mediating part of acinar cells fluid and electrolyte secretion. Indeed, deletion of NHE1 (28) and AE2 (115) in mice resulted in severe phenotypes. Although the severe phenotypes precluded using the mice to study the role of NHE1 and AE2 in exocrine glands functions *in vivo*, the knockout mice were useful in demonstrating the functional role of NHE1 and AE2 in isolated secretory cells.

TMEM16a/Ano1—It has long been recognized that Cl^- exits acinar cells by a conductive pathway (186) that was later demonstrated as the apical Ca^{2+}-activated Cl^- channel (CaCC) (17, 200, 335). The channel has been extensively characterized biophysically to be a voltage- and Ca^{2+}-activated, time-dependent outwardly rectifying channel (224, 274). However, the molecular identity of the channel eluded extensive searches until recently. Several CLC family (274) and bestrophins, in particular Best2 (186), have been suggested as the molecular identity of CaCC. However, characterization of the various expressed Best channels did not fully recapitulate the features of the native CaCC (224), and even when they closely matched these properties, like Best2, deletion in mice showed that Best2 is not the acinar CaCC (361). Recently, three independent groups have used different approaches to identify TMEM16a/Anoctamin 1 (ANO1) as the CaCC in several cell types (43, 383, 459). Moreover, TMEM16a/ANO1 is expressed at high levels in the luminal membrane of salivary glands (383, 459) and pancreatic acinar cells (166) (but not in the duct), knockdown of TMEM16a/ANO1 by siRNA reduced salivary secretion (459) and knockout in mice eliminated the CaCC activity in acinar cells (361). It was not possible to use mice with global deletion of TMEM16a/ANO1 to study its role in secretory glands function since TMEM16a/ANO1 is required for airway development and most mice die *in utero* or shortly after birth (359). Targeted knockout of TMEM16a/ANO1 is necessary to overcome this problem and to further examine the role of TMEM16a/ANO1 in acinar cells and glandular function. TMEM16a/ANO1 is expressed in acinar cells, but not the duct. Since the duct also expresses CaCC (128, 465), it is possible that the duct expresses another isoform of the TMEM16 family, which consists of 10 members (224). This is discussed further below.

Aquaporins—Transcellular ion secretion results in the obligatory osmotic water flow. Although water can cross the membrane bilayer, water flow in secretory cells is facilitated by the water channels aquaporins (AQPs). The AQPs family consists of 13 members, all of which can function as water channels, but some can transport other molecules like glycerol, urea, ions and CO_2 (425). The AQPs are involved in several cell functions, like cell adhesion, proliferation, migration and cell survival (425). Although our knowledge of the complement of AQPs expressed in secretory glands is not complete and sometime contradictory, the expression and perhaps function of several AQPs is associated with several diseases of secretory cells, like Sjögren's syndrome (80) and pancreatitis (210, 315). When present in the glands, the AQPs show highly restricted and cell specific expression pattern. For example, AQP1 is expressed in salivary glands endothelial and myoepithelial cells (16), while it is found in the human pancreatic duct (211), in centroacinar cells and in both the apical and basolateral domains of intercalated and intralobular ducts (42). However, deletion of AQP1 in mice has no effect on salivary glands function (272), yet the deletion of AQP1 in mice results in defective dietary fat processing (254) and pancreatic exocrine insufficiency in LXRβ $^{-/-}$ mice is associated with a reduction in AQP1 expression (111). This suggests that perhaps the AQPs have cell specific function in different acinar cells. Another possibility is adaptation of salivary glands to AQP1 deletion.

The established AQP in acinar cells is AQP5, which is expressed in the luminal membrane of acinar cells (80). A critical role of AQP5 in exocrine glands fluid secretion was established by knockout in mice (8, 272) and aberrant trafficking of AQP5 in Sjögren's syndrome patients (98, 372, 403). AQP5 knockout in mice reduces salivary glands secretion by more than 60% (272) and changes in cell volume in response to osmotic perturbations (317). A key regulatory mechanism of AQP5 activity is trafficking to the plasma membrane in response to cell stimulation (8). In addition, AQP5 appears to regulate the water permeability of the paracellular pathway. Deletion of AQP5 disrupted integrity of the tight junction and reduced the paracellular water permeability (98). Trafficking of AQP5 to the plasma membrane is impaired in salivary glands of patients with Sjögren's syndrome (98, 372, 403).

The salivary glands duct secretes little fluid (66, 274) and does not express AQP5 (258) or AQP1 (16). However, pointedly, gene transfer of AQP1 to the salivary duct resulted in a marked increase in salivary glands fluid secretion and increased Na^+ in the secreted fluid (112). Transduced AQP1 is expressed in the luminal membrane of the rat (283) and mini pigs salivary glands duct (115). This is reminiscent of expression of AQP1 in the pancreatic duct (210–212), which secretes most of the fluid in the pancreatic juice and does not absorb the Na^+. The simplest interpretation of these observations is that the pancreatic and salivary gland ducts have different water permeability and luminal membrane water permeability of the ducts dictates their function in secretory glands. In the salivary glands the duct mainly regulates electrolyte composition of the secreted fluid, while the pancreatic duct determines both, electrolyte composition and volume of the secreted fluid. In addition, AQP1 may reduce the function of ENaC of the salivary gland ducts to allow the net Na^+ efflux to fuel fluid secretion.

C. Model and Regulation

The available information on the localization and function of acinar cells ion transporters leads to the mechanism of acinar cells fluid and electrolyte secretion illustrated in Fig. 2. Acinar cells fluid and electrolyte secretion is fueled by the basolateral Na^+/K^+ ATPase pump, which set intracellular Na^+ at about 20 mM and intracellular K^+ at about 140 mM (64, 221, 336, 338). The basolateral NKCC1 and NHE1 are the main routes of Na^+ influx that feeds the Na^+/K^+ ATPase pump (469, 470). The basolateral Ca^{2+}-activated K^+ channels set the membrane potential at −50 to −60 mV (186, 331). NKCC1 is the major route of Cl^- influx into acinar cells and together with the Cl^-/HCO_3^- exchanger AE2 sets intracellular Cl^- at about 60 mM, which is 5 fold above electrochemical equilibrium (186, 472). NHE1 and AE2 also set cytoplasmic pH at about 7.2 (272, 282, 283, 295), which is one order of magnitude below the H^+ electrochemical equilibrium, and guard against large fluctuation in pH_i. The main transporters at the luminal membrane are the Ca^{2+}-activate Cl^- channel TMEM16A/Ano1 (224, 361) and the water channel AQP5 (80, 218, 255). The acinar cells tight junction is permeable to Na^+ and is the main route of transcellular Na^+ flux (237, 274, 404).

Acinar cells fluid and electrolyte secretion is a Ca^{2+}-initiated and regulated process that can be augmented by the cAMP/PKA system. The secretory process is initiated by an increase in $[Ca^{2+}]_i$. As outlined above, the physiological receptor-evoked Ca^{2+} signal is in the form of Ca^{2+} oscillations that initiate at the apical pole and spread to the basal pole in the form of propagated Ca^{2+} waves (196, 208, 414). Accordingly, activation of the apical Cl^- channel is the key initial step that initiates acinar cells fluid and electrolyte secretion (224, 361, 459). Once the Ca^{2+} waves arrive at the basolateral membrane, they activate the K^+ channels (294, 331, 362). Activation of the Cl^- and K^+ channels leads to Cl^- efflux into the luminal space and K^+ efflux to the interstitial space. The Ca^{2+} increase also activates AQP5 (184, 185). Hence, KCl efflux is followed by the obligatory water efflux through AQP5 to the luminal space and cell shrinkage (8, 17, 335). To compensate for the negative charge due to Cl^- secretion, Na^+ crosses from the basal to the luminal side mostly through the paracellular pathway, resulting in NaCl secretion.

Cell shrinkage has two consequences, it facilitates reduction in $[Ca^{2+}]_i$ back to baseline and it activates ion influx across the basolateral membrane. Thus, increase in cell volume facilitates and a decrease in cell volume decreases the Ca^{2+} signal (200, 289). Reduction of $[Ca^{2+}]_i$ to basal level inhibits the Cl^- and K^+ channels to temporally stop fluid and electrolyte secretion. Most importantly, cell shrinkage activates the volume-sensitive NKCC1 (137, 159), NHE1 (5) and AE2 (8). The molecular mechanism for activation of NHE1 and AE2 by cell shrinkage is not well understood, although it involves activation of several kinases, like PKC and p38 MAPK, and inhibition of PP1 (329). Cell shrinkage leads

to phosphorylation of NKCC1 by the volume sensitive SPAK kinase (76). The role of these regulatory mechanisms in secretory glands function, although likely, is yet to be established. The activated NKCC1 mediates most of the Na^+, K^+ and Cl^- uptake to restore ionic content and volume of acinar cells. The activated AE2 and NHE1 contribute about 30% of the Na^+ and Cl^-. Restoration of cell volume and ionic content prepares the cell for the next cycle of Ca^{2+}-regulated fluid and electrolyte secretion that occurs during every spike of Ca^{2+} oscillations.

The cycle of the secretory process is highly synchronized and the Ca^{2+} oscillations are closely followed by oscillation of intracellular Cl^- (and likely K^+) that are followed by oscillations in cell volume (106, 107). Moreover, synchronization extends to entire acini that function as a syncytium. The acinar syncytium is coupled by gap junctions made of connexins 26 and 32 (270).

V. DUCTAL FLUID AND HCO_3^- SECRETION

A. Overview

Exocrine secretion is a two stage process, whereby the acinar cells secret isotonic NaCl-rich fluid and the ducts modify the ionic composition and in some glands, like the pancreas, secrete most of the fluid. For long time studies of ductal function lagged behind studies of acinar cell. Contributing reasons were the fact that acini secret the protein, like digestive enzymes and mucins, or the bulk of the fluid in the case salivary glands, which were considered the central specialized function of secretory glands. In addition, the ducts comprise only between 5% (pancreas and parotid gland) to 20% (submandibular) of the gland cell volume, which limited access to the ducts. Advances in molecular, cellular and physiological techniques over the past 25 years changed this and revealed the molecular identity, function and regulation of ductal ion transporters at the basolateral and luminal membranes (274, 471). Major discoveries are the identification of anion channels in the luminal membrane, such as CFTR and CaCC; identification of the basolateral Na^+-HCO_3^- cotransporter (234, 471), which was shown to be critical for pancreatic HCO_3^- secretion (180) and cloned as pNBC1 (1) and later re-named NBCe1-B (35). A breakthrough was made with the discovery of the Cl^-/HCO_3^- exchangers SLC26 transporters (160) and the recognition of their essential role in epithelial HCO_3^- secretion (91, 215). Yet another significant finding was the discovery of HCO_3^- absorbing mechanisms like NHE3 (234, 250) and NBC3 (NBCn1-A) (250, 327) at the luminal membrane of the ducts, which suggested the ducts absorb and scavenge HCO_3^- in the resting state. The major ion transporters expressed in the luminal and basolateral membranes of the duct are summarized in Tables 1 and 2, respectively, and illustrated in Fig. 3. Expression and membrane localization of these transporters have been demonstrated in the pancreatic and salivary ducts, including the exception of ENaC, which is expressed only in the luminal membrane of salivary glands duct (65, 66). Accordingly, salivary gland ducts absorb the Na^+ from the saliva, whereas the pancreatic duct does not absorb but secrete Na^+ into the pancreatic juice.

B. Duct Cells Electrolyte Transporters

Na^+/K^+ ATPase—The Na^+/K^+ ATPase pump is abundantly expressed in the basolateral membrane of the ducts (256, 369, 399, 410). The primary Na^+/K^+ ATPase pump in conjunction with the basolateral K^+ channels uses and converts the chemical energy in ATP to osmotic energy in the form of the Na^+ and K^+ gradients and a negative membrane potential, which fuel fluid and electrolyte secretion by the duct. The Na^+ gradient is used for HCO_3^- accumulation in the cytosol and the membrane potential facilitates HCO_3^- fluxes by electrogenic transporters at the basolateral and luminal membranes.

K^+ channels—Although the activity of several K^+ channel is found in duct cells, the molecular identity of the major K^+ channel is not fully established. In the pancreatic duct MaxiK channels are the likely candidates maintaining a negative membrane potential during HCO_3^- secretion (124). These channels are activated by Ca^{2+}, have a large conductance (125–250 pS), and are encoded by the *Kcnma1* gene (30). In earlier reports MaxiK$^+$ channels were thought to be localized at the basolateral membrane of pancreatic duct cells (298). However, a recent study revealed that MaxiK channels are expressed at the luminal membrane in guinea pig pancreatic duct cells (423). Such a localization may account for the increased Ca^{2+} sensitivity of the MaxiK channels by the cAMP/PKA pathway, and contributes to the secretin-induced ductal secretion (124). In addition, activation of luminal MaxiK channels can account for part of the potentiation of ductal fluid and HCO_3^- secretion by Ca^{2+} mobilizing receptors.

Luminal localization of the MaxiK channels was also observed in salivary gland ducts (288). Such localization indicates that another K^+ channels must be expressed at the basolateral membrane of the duct. Indeed, MaxiK channels do not seem to contribute to duct cells resting membrane potential, possibly because they have very low open probability at the unstimulated state. A potential basolateral K^+ channel is Ba^{2+}-sensitive channel of 82 pS conductance, which is the main channel responsible for the resting K^+ permeability (305). In this respect, as outlined above, salivary glands express two K^+ channels, the MaxiK (294, 360) and the mIK1 channels (27, 146). The salivary glands absorb the Na^+ and secrete K^+ into the salivary fluid. Deletion of the *Kcnma1* gene coding for MaxiK had no effect on salivary glands fluid secretion (360), but impaired K^+ secretion (288), establishing the role of MaxiK as the channel that controls K^+ efflux and determines the potential of the luminal membrane. Since deletion of the *Kcnn4* and the *Kcnma1* genes in mice reduced receptor-stimulated fluid and K^+ secretion by salivary glands (362), it is likely that mlK1 is the K^+ channel at the basolateral membrane of the duct. The use of the available mice with single and combined deletion of the *Kcnn4* and *Kcnma1* genes should allow addressing this problem directly in the salivary glands and pancreatic ducts.

Na^+-HCO_3^- cotransporters (NBCs)—HCO_3^- secretion requires HCO_3^- entry at the basolateral membrane with transport characteristics adequate to maintain HCO_3^- accumulation in the cytoplasm. A search for such mechanisms identified a Na^+/H^+ exchange activity with properties of NHE1 and a Na^+-HCO_3^- co-transport (NBC) activity with the properties of what became known as NBCe1-B in the basolateral membrane of the rat pancreatic duct (470). After the initial discovery of NBC activity in the rat pancreatic duct, similar activity was demonstrated in all other species examined, including the guinea pig duct (178, 347). The basolateral NBC isoform was cloned from the pancreas and named pNBC1 (1). After identification of all members of the superfamily of Na^+-driven HCO_3^- transporters it was re-named NBCe1-B (35). NBCe1-B is expressed at the basolateral membrane of most, if not all, epithelia, including salivary gland acinar and duct cells (250).

While NHE1 functions as electroneutral exchanger, NBCe1-B is an electrogenic transporter with most likely $1Na^+$-$2HCO_3^-$ stoichiometry, although NBCe1-B stoichiometry appears to depend on the cell type in which it is expressed (131), and can be altered by PKA-dependent phosphorylation at Ser1026 (130). The electrogenic NBCe1-B uses the Na^+ gradient more efficiently than NHE1 to accumulate cytosolic HCO_3^- and indeed NBCe1-B mediates the bulk of basolateral HCO_3^- entry during ductal fluid and HCO_3^- secretion (180, 183). Significantly, NBCe1-B behaves as a $1Na^+$-$2HCO_3^-$ cotransporter when expressed in a pancreatic ductal cell line (131). Although the stoichiometry of the transport was not directly measured in native pancreatic ducts, it is considered to be $1Na^+$-$2HCO_3^-$ in the stimulated duct since it mediates HCO_3^- influx across the basolateral membrane (404), which at a membrane potential of −60 mV is possible only with a $1Na^+$-$2HCO_3^-$ stoichiometry. The

activity of NBCe1-B is regulated by the protein named IRBIT (394, 457), the mechanism and significance of which is discussed in detail bellow.

The duct also expresses an electroneutral NBC in the luminal membrane (327) that was cloned as NBC3 (343) and later re-named NBCn1-A. The finding of Na^+ and HCO_3^- absorbing mechanisms at the luminal membrane of the duct was unexpected. Similar to the case of NHE3 (see below) (3), NBCn1-A (327) is regulated by CFTR in a cAMP-PKA-dependent manner. Stimulation of CFTR with PKA leads to inhibition of NHE3 and NBCn1-A (3, 327). NHE3 (150) and perhaps NBCn1-A (35) are also activated by IRBIT (see below). Based on their regulation by IRBIT and stimulated CFTR, we proposed that NHE3 and NBCn1-A are part of a HCO_3^--regulating complex in the duct, which serves as a HCO_3^- salvage mechanism at the resting state to maintain acidified pancreatic juice (118, 263) and saliva (250).

Cystic fibrosis transmembrane conductance regulator (CFTR)—CFTR was discovered as the protein mutated in cystic fibrosis (199, 356, 364). Since its discovery, CFTR became one of the most extensively studied protein and serves as a model to understand the function of proteins of similar structure and function. CFTR (ABCC7) belongs to the ATP-binding cassette (ABC) transporters superfamily. Most ABC transporters function as membrane pumps for organic molecules, which transport their substrates against the electrochemical gradient using energy generated from ATP hydrolysis (74). Unlike other ABC transporters, CFTR has anion channel activity that conducts the substrate molecule down the electrochemical gradient. In expression cloning, CFTR functions as a small conductance (5–10 pS) Cl^- channel with a linear current-voltage (I-V) relationship that is activated by the cAMP/PKA pathway (411). Channels with similar properties have been identified in the pancreatic and salivary gland duct cells of many species, including humans. The presence of CFTR in the luminal membrane of the pancreatic and salivary glands ducts has now been firmly established in many species by immunohistochemistry (49, 404, 466)

CFTR functions as a Cl^- channel with limited permeability to HCO_3^- at normal intra and extracellular Cl^- (245, 340, 387). Based largely on computer modeling, it has been suggested that CFTR may function as a HCO_3^- channel in the pancreatic duct (181, 312, 353, 387, 437). However, several key findings indicate that CFTR-mediated HCO_3^- flux has limited and defined role in ductal HCO_3^- secretion. First, the ducts express luminal, DIDS-sensitive Cl^-/HCO_3^- exchange activity (235, 424, 471) that is mediated by the luminal SLC26 transporters (405, 433). Second, CFTR is regulated by extracellular and intracellular Cl^- and at extracellular Cl^- of higher than 30 mM it does not transport significant amount of HCO_3^- (387, 448). Third, Cl^- has to be removed from the luminal space for the duct to secrete HCO_3^- through CFTR (181, 325). Forth, deletion of Slc26a6 impairs ductal fluid and HCO_3^- secretion (433). Hence, although having a HCO_3^- selective channel at the apical membrane of duct cells it is theoretically possible to secrete 200 mM HCO_3^- at a membrane potential of −60 mV, the CFTR P_{HCO3}/P_{Cl} is between 0.2–0.5 in pancreatic duct cells when measured with symmetrical Cl^- solutions (312, 400). With this permeability ratio, the CFTR anion channel secretes Cl^- much faster than HCO_3^-, thus CFTR would be unable to secrete sufficient HCO_3^- to account for the bulk of the secreted HCO_3^-. Finally, fluid secretion requires osmotic HCO_3^- secretion. Strict exchange of HCO_3^- for Cl^- by CFTR will not result in osmotic solute secretion.

Nevertheless, CFTR-mediated HCO_3^- flux can become important at the distal portion of the ducts to determine the final HCO_3^- concentration in the secreted fluids. A unique form of regulation of CFTR activity discovered recently is by the WNK/SPAK pathway. The WNK kinases were discovered in a search for MAPK homologues and the family consists of four

members with conserved kinase domain, but diverse N and C termini (reviewed in (164)). Interest in the WNKs increased greatly with the discovery that mutations in WNK1 and WNK4 cause hypertension in humans (442). The WNKs act mostly by regulating surface expression of Na^+, K^+ and Cl^- transporters (194), through regulation of their endocytosis (165). Subsequent work revealed that many functions of the WNK are mediated by the downstream oxidative stress-responsive kinase 1 (OSR1) and STE20/SPS1-related proline/alanine-rich kinase (SPAK) (reviewed in (77)). The WNK/SPAK kinase pathway appears to have dual function in the ducts. At normal physiological $[Cl^-]_i$ the WNK kinases reduce surface expression of CFTR expression (455, 456). In the pancreatic and salivary glands the WNKs act through SPAK to control the activity of NBCe1-B and CFTR, and knockdown of the WNKs and SPAK increases pancreatic duct fluid and HCO_3^- secretion (456). However, at low cytoplasmic $[Cl^-]_i$, as occurs in the distal duct, the WNK/SPAK pathway appears to have an opposite role. The CFTR HCO_3^- permeability appears to be dynamically regulated by intracellular Cl^- (325). During pancreatic secretion, $[Cl^-]_i$ can drop to as low as 5 mM. This low $[Cl^-]_i$ in turn activates WNK1 and the downstream OSR1 and SPAK. Activation of the WNK1/OSR1/SPAK pathway under this $[Cl^-]_i$ conditions resulted in a dramatic increase in CFTR HCO_3^- permeability, making CFTR primarily a HCO_3^- channel (325). Function of CFTR as a HCO_3^- channel at the distal duct can be essential for the secretion of pancreatic juice containing 140 mM HCO_3^-. Hence, it appears that under resting conditions and at the proximal duct the WNK/SPAK pathway reduces NBCe1-B and CFTR activity to stabilize the resting state and perhaps minimize HCO_3^- fluxes by CFTR. On the other hand, at the distal duct an important function WNK/SPAK pathway is to switch CFTR from primarily a Cl^- channel to a HCO_3^- channel to set the final HCO_3^- concentration in the secreted fluids.

In addition to Cl^- and HCO_3^- channel activity, CFTR functions as a central regulator of ductal fluid and electrolyte secretion by virtue of regulating the function of many transporters at the luminal membrane of the ducts. The central role of CFTR in ductal function is well exemplified by the aberrant fluid and electrolyte transport and pancreatic insufficiency seen in CF patients (94). CFTR exists in a macromolecular complex at the luminal membrane of secretory epithelia, which is assembled with the aid of scaffolding proteins. The three amino acids at the C-terminal end of CFTR form a PDZ ligand that binds to PDZ domains containing scaffolds in epithelia (395, 431). In addition, CFTR interacts with SNARE proteins, AKAPs, kinases and phosphatases (135). In the complexes, CFTR directly or indirectly regulates the activity of several transporters. Functional interactions with CFTR were reported for ENaC, outwardly rectifying Cl^- channels, Ca^{2+}-activated Cl^- channels, ROMK2 and KvLQT1 K^+ channels, the SLC26 transporters, NBCn1-A (NBC3) and perhaps aquaporins (222, 237). Below we discuss the significance of several of these interactions for ductal secretion.

Ca^{2+}-activated Cl^- channels (CaCCs)—Ca^{2+}-activated chloride channel activity (CaCCs) is present in the luminal membrane of duct cells (125, 128, 424, 465). The molecular identity of ductal CaCC is still unknown. The discovery of the TMEM16/Anoctamin (ANO) family as the CaCC in acinar cells (43, 383, 459) suggest that the ductal CaCC is likely a member of this family. However, immunostaining shows that TMEM16A/ANO1 is expressed in the luminal membrane of acinar cells and that the duct does not express TMEM16A/ANO1 (383, 459). To date, several members of the TMEM16/ANO family were shown to function as Cl^- channels, including TMEM16B/ANO2, TMEM16G/ANO7, TMEM16J/ANO10 and perhaps TMEM16F/ANO6 (406). Human and rodent pancreata and the salivary duct express mRNA for several members of TMEM16/ANO family, including TMEM16B/ANO2, TMEM16F/ANO6 and TMEM16J/ANO10 (MGL and SM, unpublished observation). Although TMEM16F/ANO6 was reported recently to function as a phospholipid scrambler (410), it may also function as Cl^- channel. It will be

interesting to determine how the two activities of TMEM16F/ANO6 are related. Whether the other TMEM16/ANO isoforms also mediate ductal CaCC awaits further studies.

Interest in the ductal CaCC stems from the possibility that it may replace the Cl^- channel function of CFTR in HCO_3^- secretion. Studies in the human pancreatic duct cell line PANC1 (476), raise questions as to the feasibility of such a role. Moreover, an increase in $[Ca^{2+}]_i$ alone by stimulation of the cholinergic and CCK receptors does not evoke significant fluid and HCO_3^- secretion by the pancreatic duct (460) and cholinergic simulation of salivary glands result in low HCO_3^- secretion (45). The P_{HCO3}/P_{Cl} of heterologously expressed TMEM16A is 0.1–0.5 at $[Ca^{2+}]_i$ levels normally induced by receptor stimulation. Interestingly, the P_{HCO3}/P_{Cl} permeability of TMEM16A appears to be regulated by $[Ca^{2+}]_i$ (MGL, unpublished observation). The physiological role of this finding in epithelial HCO_3^- secretion is not known at present.

Cl^-/HCO_3^- exchangers: AEs and the SLC26 transporters—The pancreatic duct secretes most of the fluid in the pancreatic juice. The copious fluid secretion must involve large net transcellular salt transport. Since the pancreatic duct absorbs the Cl^- and secretes fluid containing Na^+ and HCO_3^-, and since Na^+ secretion is largely paracellular, it follows that pancreatic duct HCO_3^- secretion must be an active process and a $1Cl^-/1HCO_3^-$ exchange, whether by coupled or uncoupled transport, cannot lead to net electrolyte secretion necessary to drive fluid secretion. This puts a thermodynamic constrain as to the nature of the Cl^- absorbing and HCO_3^- secreting mechanism that must function with a stoichiometry of $HCO_3^-/Cl^- > 1$. We will argue below that SLC26 transporter Slc26a6 fulfill this requirement and is critical for pancreatic duct fluid and electrolyte secretion.

Because the absence of CFTR activity in CF results in acidic pancreatic juice, it was assumed that CFTR directly mediates the tightly coupled Cl^- absorption and HCO_3^- secretion or function in concert with Cl^-/HCO_3^- exchange to mediate HCO_3^- secretion by the duct (49, 169, 404). An early model for pancreatic HCO_3^- secretion suggested that electroneutral Cl^-/HCO_3^- exchanger in the apical membrane mediates HCO_3^- secretion in conjunction with Cl^- channel that cycles the Cl^- (237, 400, 401, 404). Subsequently, it was revealed that CFTR mediates the apical Cl^- channel activity and that the activity of the apical Cl^-/HCO_3^- exchanger is dependent on the expression of CFTR (235, 238). Furthermore, CFTR mutations associated with pancreatic insufficiency exhibited severe defect in CFTR-dependent Cl^-/HCO_3^- exchange activity (61). These findings prompted extensive search to identify the molecular nature and function of the ductal luminal Cl^-/HCO_3^- exchangers.

The first family of Cl^-/HCO_3^- exchangers to be considered are members of the solute-linked carrier 4 (SLC4) family (363). The ducts do express the SLC4 transporter AE2 (SLC4A2), but it is located on the basolateral membrane of pancreatic and salivary gland duct cells (370, 372). AE2 together with NHE1 is essential for regulation of pH_i and protects the cells against an alkali load, but does not appear to play a major role in transcellular ductal HCO_3^- transport. A breakthrough in our understanding of luminal Cl^-/HCO_3^- exchange in epithelia was made with the discovery that the protein previously identified as down regulated in adenoma (DRA), is a Cl^- transporter highly expressed at the luminal membrane of the colon, and mutation in which lead to the disease congenital Cl^- diarrhea (160, 281). Shortly thereafter it was shown that DRA functions as a Cl^-/HCO_3^- exchanger (273). With the renaming of the family it is designated as SLC26A3 and is expressed in several epithelia.

The SLC26 transporters (SLC26Ts) family consists of 11 genes and 10 members (Slc26A10 is pseudo gene) with several members associated with human diseases (for review see (91)).

The family members have diverse functional properties (314), with SLC26A1 and SLC26A2 functioning as SO_4^{2-} transporters (262), Slc26a3 and Slc26a6 as electrogenic Cl^-/HCO_3^- exchangers (214, 449), Slc26a4 as electroneutral $Cl^-/HCO_3^-/I^-$ exchanger (389), SLC26A5 functions as an anion regulated, voltage sensing motor protein (376), and SLC26A7 (204) and SLC26A9 (90) function as *bona fide* Cl^- channels. The function of SLC26A11 is not known at present. Pancreatic and salivary gland ducts express several members of the family, including the ubiquitous SLC26A2 and SLC26A11 and Slc26a6 (215, 314), with salivary glands also expressing Slc26a4 (389). Notably, Slc26a3 functions as an electrogenic Cl^-/HCO_3^- exchanger with a $2Cl^-/1HCO_3^-$ stoichiometry (215, 388), while Slc26a6 functions as a $2HCO_3^-/1Cl^-$ exchanger (209, 388). Slc26a3 and Slc26a6 can also mediate uncoupled anion currents, with the mode of transport determined by a glutamate residue that is conserved in all species and in all SLC26Ts (313).

Slc26a6 is the major Cl^-/HCO_3^- exchanger in the salivary and pancreatic duct and is essential for fluid and HCO_3^- secretion by the ducts (389, 405, 433). SLC26A6 was originally identified in a search for novel SLC26Ts (247), and as the oxalate transporter in the renal proximal tubule (209). Oxalate handling by Slc26a6 mediates oxalate homeostasis by secreting oxalate into the intestinal lumen, and deletion of Slc26a6 results in urolithiasis due to increased renal oxalate load (189). Oxalate transport by Slc26a6 is not likely to be relevant for the pancreatic duct, but may have a role in sialolithiasis (142). An important feature of the SLC26Ts and CFTR is their mutual regulation. Thus, the STAS domain at the C terminus of the SLC26Ts interacts with the R domain of CFTR and the interaction is required for activation of both the SLC26Ts and of CFTR (215). This mode of regulation is discussed further below.

Na^+/H^+ exchangers (NHEs)—The NHEs are electroneutral $1Na^+/1H^+$ exchangers. Phylogenetic analysis indicates that the mammalian alkali cation/proton gene family (the solute carrier SLC9A or NHE family) is comprised of three general gene clusters: (a) five plasma membrane-type Na^+-selective NHEs (NHE1-NHE5); (b) four organellar cation non-selective NHEs (NHE6-NHE9); and (c) two distantly related NHE-like genes, termed Na^+/H^+ antiporter 1 (NHA1) and NHA2, that have closer homology to the fungal/plant NHA1 and bacterial NhaA (36, 318). The ubiquitous housekeeping NHE1 is essential for pH_i homeostasis (28), and is localized at the basolateral membrane in duct cells (151, 234, 250, 326, 370). In addition, NHE1 can provide a portion of HCO_3^- influx across the basolateral membrane during HCO_3^- secretion. However, NHE1 contribution to HCO_3^- influx must be minor, since inhibition of NHE1 by amiloride has minimal effect on secretin-induced fluid and HCO_3^- secretion by the pancreatic duct in most species (422, 445).

Interestingly, NHEs are also expressed in the luminal membrane of salivary and pancreatic ducts (250, 326), which may salvage HCO_3^- when HCO_3^- secretion is not required. Pancreatic juice at resting or low flow rates is acidic and contains high level of CO_2, indicating that an active H^+ secreting mechanism is functional at this state (118, 263). NHE2 and NHE3 are localized at the luminal membrane of the large-sized mouse pancreatic and salivary glands ducts (33, 234, 250, 264, 326). The role of NHE2 is not clear since deletion of NHE2 in mice had no obvious disease phenotype or altered pH homeostasis and HCO_3^- metabolism in several organs expressing NHE2 (384), including the pancreatic (234) and salivary glands ducts (98, 250). On the other hand, deletion in mice showed that NHE3 is functional in the luminal membrane of the duct (3). Notably, the luminal NHE3 is associated with CFTR with the aid of PDZ-containing scaffolding proteins and its activity is regulated by CFTR in the HCO_3^- transporting complex (3). In addition, the activity of NHE3 is regulated by IRBIT (149, 150) (see below).

V-type H^+ pump and H^+/K^+ ATPase pumps—Early studies in pigs suggested that a vesicular V-type H^+-ATPase pump can function as a HCO_3^- loading mechanism at the basolateral membrane of the duct (428). These studies relied mainly on pharmacological tools that may have not reflected the function of the V-type H^+ pump. In this respect, no effect of H^+ pump inhibition was found on fluid and HCO_3^- secretion in the guinea pig duct (183). The salivary glands duct was reported to express V-type H^+-ATPase pump that shifts from intracellular organelles to the luminal membrane upon chronic acidosis (374). However, no functional evidence is available to show participation of the pump in acid (rather than HCO_3^-) secretion in salivary ducts.

The topic of ductal H^+ pumps was revisited very recently in the rat pancreatic duct to conclude the function of two H^+/K^+ ATPase pumps in the duct, the gastric and the non-gastric type pumps (309). Immunolocalization suggested that both H^+/K^+ ATPase pumps are expressed in the lateral and luminal membranes. Based on inhibitor studies, the H^+/K^+ ATPase pumps were shown to mediate modest H^+ clearance from the cytosol of acidified duct cells (309). However, inhibition of the H^+/K^+ ATPase pumps with omeprazole and SCH-28080 strongly inhibited secretin-stimulated ductal fluid secretion (309). Inhibition of fluid secretion by inhibition of the H^+/K^+ ATPase pumps appears to be the strongest evidence for their expression, localization and function in the duct. Functioning of H^+/K^+ ATPase pumps in the basolateral membrane of the duct can be of particular significance since pumps can generate very large gradient using the energy in ATP. The gastric H^+/K^+ ATPase pump generates the steepest ion gradient in mammalian cells of six orders of magnitude (393). Such a pump in the basolateral membrane can function with NBCe1-B to markedly enhance HCO_3^- loading of the duct during stimulation of fluid and HCO_3^- secretion. However, this topic needs significantly additional extensive studies *in vivo* and *in vitro* using the available mice with targeted disruption of the H^+/K^+ ATPase pumps to establish and better understand the function of these pumps in pancreatic and salivary glands ductal function.

Aquaporins—Since the paracellular pathway is permeable to water, early models of epithelial fluid secretion assumed that water follows down its osmotic gradient from the basolateral to the luminal side via the paracellular pathway. In fact, significant portion of the water secreted by salivary glands acini does flow through the paracellular pathway (144, 285), including in salivary glands of AQP5-/- mice (198). However, water flow through the paracellular pathway cannot account for most of the salivary glands water flow. It is now clear that significant portion of water transport is mediated by the water channels aquaporins (AQP) and is a regulated process. There are at least 13 AQP genes (AQP0-AQP12) in mammalian cells (426). Although several members of the AQP family have been reported to be expressed in the exocrine pancreas (170), AQP1 and AQP5 seem to be the major AQPs in the pancreatic duct. Immunolocalization indicates expression of AQP1 at both the basolateral and luminal membranes, and AQP5 at the luminal membrane of pancreatic duct cells (42, 211, 212). Most notably, the salivary glands duct does not express AQP1, but when transduced with AQP1 in the rat (24, 79) and mini-pig (112) it secretes large volume of fluid, highlighting the function and importance of AQP1 in the luminal membrane for fluid secretion by the duct.

The Epithelial Na^+ channel ENaC—Like many other epithelia, the salivary duct expresses the epithelial Na^+ channel ENaC in the luminal membrane (49, 65). ENaC appears to be the major Na^+ absorbing pathway in the salivary glands duct. Deletion of both NHE2 and NHE3 had no effect on ductal Na^+ absorption, but inhibition of ENaC by amiloride inhibited both Na^+ and Cl^- absorption by the duct in stimulated glands (49). However, these findings may indicate that the NHEs' major role is in HCO_3^- salvage in the resting state, since they are inhibited in the stimulated state when CFTR is activated (3, 234). ENaC is

extensively regulated by multiple physiological pathways, as expected from its central function in salt-sensitive epithelia (228). ENaC activity can be regulated acutely during cell stimulation. In the salivary glands duct, stimulation of luminal and basolateral P2Y2 receptors inhibits ENaC activity by a pertussis toxin-sensitive and pertussis toxin-independent pathways (311). The precise mechanism by which P2Y2 receptors regulate ENaC is yet unknown, although it may involve in part depletion of the lipid PIP_2, which activates ENaC (223, 464).

Of particular physiological significance is the regulation of ENaC by receptors that stimulate the glucocorticoid-induced kinase 1 (Sgk1), including mineralocorticoids, such as aldosterone (228). Regulation of ENaC by Sgk1 is biphasic. In the first phase that is completed in about 30 min, activated Sgk1 increases surface expression of the Na^+/K^+ ATPase pump in the basolateral membrane and of ENaC in the luminal membrane without affecting their transcription and translation. In the second phase that requires 6–8 hrs, Sgk1 increases transcription and translation of ENaC (228). ENaC surface expression is regulated by a well defined endocytotic mechanism that involves the ubiquitin ligase Nedd4-2 (367). Nedd4-2 regulates ENaC by interaction of Nedd4-2 WW domains with the proline-rich (PY) motifs (PPPxYxxL) located in the C-termini of the α, β, and γ subunits of ENaC (141). Nedd4-2 then polyubiquitinates the ENaC subunits to cause their internalization by a clathrin-coated vesicle endocytosis (430). Nedd4-2 appears to interact mostly with mature ENaC and the interaction requires their localization in caveolae and interaction of Nedd4-2 and ENaC with caveolin-1 (229). The key regulation of ENaC expression and activity by Nedd4-2 was recently demonstrated *in vivo* by showing that deletion of Nedd4-2 in the lung markedly increased ENaC activity and airway Na^+ absorption to reproduce the lung phenotype observed in cystic fibrosis (206). However, whether this occurs in cystic fibrosis remains controversial since the lung of the porcine cystic fibrosis model does not show altered ENaC activity and Na^+ absorption (53).

Regulation of ENaC by Sgk1 involves the phosphorylation of Nedd4-2 by Sgk1 on specific residues. The phosphorylated Nedd4-2 is then sequestered by the scaffolds 14-3-3 to prevent its interaction with and consequently inhibition of ENaC (228). Phosphorylation of Nedd4-2 by Sgk1 is regulated by the WNK kinases, where the WNKs function as scaffolds to recruit both Nedd4-2 by Sgk1 to allow Nedd4-2 phosphorylation and ENaC surface insertion (155). This system is functional in the salivary glands duct, since modulation of Nedd4-2 and Sgk1 activities have the expected effect on ENaC function (85, 351). Furthermore, treatment of rats with dexamethasone and low Na^+ diet resulted in marked upregulation of β and γ ENaC expression (MGL and SM, unpublished observations). In addition, ENaC and CFTR appear to affect the activity of each other, but in a mechanism that appears to be different from that in the lung (29, 49). In the lung, CFTR is proposed to inhibit ENaC channel function and deletion of CFTR may increase ENaC activity, which can be clearly demonstrated in an *in vitro* models (29). However, in salivary glands deletion of CFTR inhibited Na^+ absorption by ENaC and inhibition of ENaC function markedly inhibited CFTR-dependent Cl^- absorption (49). Deletion of CFTR resulted in almost elimination of α ENaC expression (49). This likely explains the marked reduction in ENaC activity in CF patients sweat duct (352). It will be of particular interest to determine expression of CFTR and ENaC in different tissues in the various CF models.

C. Diversity of Other Channels and Transporters

Several transporters show organ and species specific expression and in these cases limit the conclusions to the specific organ and species. The pancreatic duct is unique among absorbing and secretory epithelia, including the salivary glands duct, in that it does not express ENaC, and thus it does not absorb Na^+. In fact, the pancreatic duct secretes Na^+,

which passes paracellularly (14, 404). Paracellular Na^+ secretion is essential for fluid secretion by the duct not only to maintain electroneutrality, but also as an osmolyte.

NKCC1 (SLC12A2) shows highly species specific expression. It is expressed at the basolateral membrane in a variety of Cl^- secreting epithelia and it is expressed in the rat and mouse pancreatic duct (103), but not in the pig and guinea pig pancreatic duct (103, 133) or the salivary glands duct (99, 465). NKCC1 maintains $[Cl^-]_i$ higher than its equilibrium concentration, and hence facilitates Cl^- secretion when apical Cl^- channels are opened. This may account for the relatively high Cl^- concentration of the secretin-stimulated rat and mouse pancreatic juice and perhaps the lack of pancreatic phenotype in the cystic fibrosis mice models CFTR-/- and ΔF/ΔF. The lack of NKCC activity in pig and guinea pig pancreas (103, 133) (and likely human pancreas), may explain the severe pancreatic phenotype in human with CF (441) and pigs with deleted CFTR (53). As discussed below, a low ductal $[Cl^-]_i$ is critical for producing pancreatic juice with the final high concentration of HCO_3^-. The lack of NKCC1 in the pig and human pancreas may serve this purpose to allow the high HCO_3^- concentration in their pancreatic juice.

VI. REGULATORY PROTEINS OF EPITHELIAL HCO_3^- SECRETION

A. PDZ-Based Adaptors

HCO_3^- transport by the duct is mediated by multiple transporters that are assembled into complexes by adaptor proteins containing PDZ domains. PDZ domains were identified as a conserved domain in three proteins, Post-Synaptic-Density-95, Disc-large, and Zonula Occludens-1. The PDZ domain is a protein-protein interaction module consisting of 80–90 amino acids which typically binds to target proteins harboring specific C-terminal sequences called PDZ-binding motifs or ligands (201). PDZ domains have two major functions. 1) They anchor protein, including integral membrane proteins such as receptors, transporters, channels and adhesion proteins, through their PDZ-binding motifs and 2) they bind to the PDZ domains of other PDZ proteins, thus forming scaffolding networks mediated by homo- and heteromultimers (117). Initially, PDZ-based adaptors were known to play an important role in the efficiency and fidelity of synaptic transmission in neurons (201). Subsequent studies revealed that epithelial cells express a battery of adaptors with PDZ domains and these proteins perform critical roles in the transepithelial fluid and electrolyte transport. The role of PDZ-based adaptors in ductal secretion is illustrated in Fig. 4.

The first family of PDZ proteins shown to be involved in epithelial transport is the Na^+/H^+ exchanger regulatory factor (NHERF) family. NHERF1 (EBP50), NHERF2 (E3KARP), and NHERF3 (CAP70, PDZK1) facilitate the PKA-dependent phosphorylation and membrane trafficking of CFTR and NHE3 in epithelial cells, including duct cells (89, 435). By virtue of expressing multiple PDZ domains, these adaptors assemble a large protein complex in the apical membrane in which CFTR functions as a central regulator of secretory epithelia. The requirement of CFTR and the SLC26 transporters for fluid and HCO_3^- secretion by duct cells raised the question of how CFTR and the SLC26 transporters communicate to regulate HCO_3^- secretion. The answer was provided by the discovery of the interaction and reciprocal regulation of CFTR with the ductal HCO_3^- secretory machinery. Particularly, the interaction and mutual regulation of CFTR with the HCO_3^--secreting SLC26 transporters (SLC26Ts) is mediated by the phosphorylated CFTR R domain and the SLC26Ts STAS domain and is enhanced by the interaction of CFTR and the SLC26Ts with PDZ scaffolding proteins (213). In addition, CFTR is involved in the regulation of the luminal HCO_3^- absorbing transporters NHE3 and NBCn1-A by forming a protein complex via adaptors with multiple PDZ domains such as NHERF1 and NHERF2 (3, 250, 327). As a result, the cAMP/PKA pathway activates CFTR and apical Cl^-/HCO_3^- exchange and inhibits NHE3 and NBCn1-A (89, 435). Assembling a complex with a large number of transporters with the aid

of PDZ adaptors greatly enhances signaling efficiency and fidelity of the cAMP/PKA pathway in confined regions of the luminal membrane. Upon stimulation with cAMP, CFTR activates the HCO_3^--secreting and at the same time inhibits the HCO_3^--absorbing transporters to optimize fluid and HCO_3^- secretion.

In addition to the NHERFs, duct cells express several other scaffolds with PDZ domains, such as Shank2, S-SCAM, SAP97, and PSD-95 (116, 202). The Shank family of proteins was discovered as molecular scaffolds in neuronal cells, where they serve as coordinators of membrane and cytoplasmic protein complexes in the postsynaptic density (PSD) (244, 391). The Shanks contain multiple protein-protein interacting domains, including ankyrin repeats, an SH3, a PDZ, a long proline-rich region and a sterile α motif (SAM) (391). Currently, there are three known members of the Shank family, Shank1-3. Among them, Shank2 localizes at the apical pole of pancreatic duct cells and modulates the activity of CFTR and NHE3 (140, 202). Expression of Shank2 upregulates the membrane expression of CFTR and NHE3, and this appears to be related to activation of Rho small GTPases by recruiting βPix (PAK-interacting exchange factor), a guanine nucleotide exchange factors (GEF) for the Rho small GTPases (232). By contrast with NHERF proteins that deliver cAMP/PKA signals to CFTR and NHE3, Shank2 mediates an inhibitory effect on the cAMP/PKA pathway. A detailed examination of binding partners of Shank2 revealed that Shank2 associates with phosphodiesterase 4D (PDE4D) that hydrolyzes cAMP, hence lowering local cAMP concentration in the apical microdomains (231). These findings revealed a unique form of regulation, whereby opposite signals can be delivered to the same PDZ-binding motif of a given protein by different adaptors, serving as a homeostatic system to regulate intensity of the stimulated state (231).

Notably, most ductal GPCRs have PDZ ligands at their C-termini (see fig. 4). This recruits the GPCRs to the transporting complex resulting in polarized expression of GPCRs (239, 240, 357, 392) and delivery of the second messengers to the ion transporting microdomain. This should allow confined and better controlled delivery of the stimulus. A good example for such an arrangement is of the VIP receptor $VPAC_1$, which binds through its PDZ ligand to the synaptic scaffolding molecule (S-SCAM), also known as membrane-associated guanylate kinase inverted-2 (MAGI-2) (116). S-SCAM/MAGI-2 associates with E-cadherin, a key protein at the adherens junction, in a β-catenin dependent manner (408) and recruits $VPAC_1$ to the junctional area at the apical pole of epithelial cells. Thus, S-SCAM/MAGI-2 confines $VPAC_1$ to the junctional domain to restrict VIP-induced cAMP signaling to activate CFTR at a microdomain in the vicinity of the apical pole. This, in turn, enables efficient electrolyte and fluid secretion in response to VIP with minimal effects on the cell interior (116). Fig. 4 lists the many ductal HCO_3^- transporters and GPCRs that have a typical PDZ ligand at their C-termini.

B. With-no-lysine (WNK) and Sterile 20 (STE20)-like Kinases

Recently, two families of protein kinases, the WNK kinases and the sterile 20 (STE20)-like kinases, have emerged as osmotic sensors that modulate the activity of diverse ion transporters (13, 194, 355). The family of the WNKs was discovered by search for a MAPK homologues (450) and were found to lack the conserved lysine in the kinase catalytic site. Crystal structure of the kinase domain showed the lysine is contributed by the β2, rather than the β3 strands (277). The family consists of four large members with only the kinase domain highly conserved (164). The WNKs gained prominence with the seminal discovery that mutations in WNK1 and WNK4 lead to hypertension (442). Subsequent extensive studies of their function showed that WNK1, WNK3 and WNK4 function mainly to reduce the surface expression of various Na^+, K^+ and Cl^- transporters, including NKCC1, NKCC2, NCCT, KCCT, ENaC, ROMK and Cl^- channels (164, 165, 194, 355), including CFTR (455, 456) and Slc26a6 (193). The WNKs reduce transporters surface expression by promoting

their endocytosis (148, 165). In most cases examined, the WNKs do not act directly on the ion transporters, but act on the downstream kinases, the STE20-like kinase SPAK and the oxidative stress-responsive kinase 1 kinase OSR1. The WNKs can phosphorylate the SPAK/OSR1 on T233, which activates SPAK/OSR1 and the activated SPAK/OSR1 phosphorylates the ion transporters to reduce their surface expression (355). In several cases, the kinase function of the WNKs was not required for regulation of the ion transporters. In these cases the WNKs act as scaffolding proteins that bind SPAK/OSR1 to recruit it to the transporters (13, 148, 155, 164, 456). The scaffolding function of the WNKs resides in their first 119 residues (148, 155, 456). The human mutations in WNK1 and WNK4 result in inhibition of NKCC2, NCCT and ENaC endocytosis to enhance their surface expression in the kidney, leading to excessive Na^+ absorption and hypertension (165, 194, 355).

WNK1 (325, 442, 456), WNK3 (456), WNK4 (442, 456), SPAK and OSR1 (325, 456) are abundantly expressed in ductal systems, including the pancreatic (325, 456) and salivary glands ducts (456). Recent studies from our laboratories revealed that the WNK/SPAK pathway appears to have dual roles in regulating ductal function at the resting and stimulated ducts. The WNK/SPAK pathway stabilizes the ductal resting state by prominently reducing the surface expression of all ductal transporters involved in fluid and HCO_3^- secretion (456). Thus, the WNKs act as scaffolds in which only a short segment of the WNKs N terminal domain that lacks kinase function (155, 456) binds and recruit SPAK to the Na^+-HCO_3^- co-transporter NBCe1-B (pNBC1) and CFTR to reduce their surface expression and dramatically reduce their activity at the plasma membrane (456). The WNK/SPAK pathway also reduces the surface expression (325) and activity (193, 325) of the ductal SLC26 transporters. Moreover, knockdown of the WNKs increases the activity of ductal NBCe1-B, CFTR and stimulated fluid secretion (456). The prominent inhibition of ductal fluid and electrolyte secretion by the WNK/SPAK pathway suggests that the WNK/SPAK pathway stabilize the resting state of the duct.

Regulation of the WNK/SPAK/OSR1 kinase activity is poorly understood and for the most part it is not known how these kinases are activated by stimulation of cell surface receptors or during changes of cellular activity. One well established exception is regulation of the WNK/SPAK/OSR1 kinases by osmotic stress. The WNK kinases, including WNK1, are activated by osmotic stress such as that caused by a decrease in $[Cl^-]_i$. The activated WNK1 then phosphorylates and activates the SPAK and OSR1 kinases (355). Indeed, when the human pancreatic duct cell line PANC1 and guinea pig ducts are exposed to low external and intracellular Cl^-, the WNK1-SPAK/OSR1 pathway affects the function of two apical HCO_3^- transporters (325). First, the WNK1-SPAK/OSR1 increases CFTR Cl^-/HCO_3^- selectivity to increase the CFTR HCO_3^- permeability. Second, the WNK1-SPAK/OSR1 inhibits the apical Cl^-/HCO_3^- exchange by the SLC26 transporters. When occurring in the terminal portion of the stimulated pancreatic duct, the two functions will result in pancreatic juice containing HCO_3^- at a concentration greater than 140 mM.

C. IRBIT (inositol-1,4,5-triphosphate (IP_3) receptors binding protein released with IP_3)

IRBIT was discovered several times in different contexts (83, 457), most recently in a search for proteins that interact with the IP_3-binding domain of the IP_3 receptors (IP_3Rs) (11). IRBIT competes with IP_3 for interaction with N terminal domain of IP_3Rs to play important role in the function of the IP_3Rs and Ca^{2+} signaling (10, 82). Subsequent search for proteins that interact with IRBIT discovered that IRBIT interacts with and regulates the activity of the Na^+-HCO_3^- co-transporter NBCe1-B (394). There are two highly homologous IRBIT isoforms, a short and a long IRBIT, with the long IRBIT having an N terminal extension (12). The domains common to short and long IRBITs are an N terminus PP1 anchoring site that is followed by a PEST domain (81, 83), a coiled-coil domain and a PDZ ligand at the end of the C terminus (83). The PEST domain has multiple phosphorylation sites (10), with

phosphorylation of key residues, including S68, S72 and S74, that are required for all known IRBIT functions, including regulation of IP_3Rs function (10, 82) and activation of ion transporters (394, 458). Long IRBIT has an N terminal extension rich in prolines and alanines, which differentiates the roles of short and long IRBIT functions (12).

In the duct IRBIT accumulates at the apical pole (458), a site of expression of high level of IP_3Rs, but IP_3Rs and thus IRBIT are also present in the basal pole (240). At the apical pole IRBIT regulates CFTR (456, 458), and possibly NHE3 (150) and NBCn1-A (35), while at the basal pole IRBIT regulates NBCe1-B (394, 456, 458). The NBCs are members of the superfamily of Na^+-coupled HCO_3^- transporters (NCBT), which includes the electrogenic NBCe1-A~C and the electroneutral NBCn1-A~H (35). The domain structure and function of the NCBTs are discussed in several recent reviews (35, 344). Notably, the first 45–62 residues of N terminus of several NBC, including NBCe1-B and NBCn1-A, forms an inhibitory domain and its deletion results in marked activation of the relevant NCBTs (268). Importantly, IRBIT interacts with the N terminal domain of NBCe1-B to markedly activate NBCe1-B (394). Activation of NBCe1-B by IRBIT is mediated by the PEST domain (394, 458) and required the PDZ ligands to assemble the IRBIT-NBCe1-B complex (458). These findings suggest that IRBIT induce a conformational change in NBCe1-B to dissociate its N terminal inhibitory domain from the transmembrane domain mediating the ion transport. Most notably, IRBIT activates the native NBCe1-B in pancreatic (456, 458) and salivary gland ducts (456).

Activation of NBCe1-B by IRBIT indicates that IRBIT should have a prominent role in the regulation of epithelial HCO_3^- secretion. Indeed, knockdown of IRBIT markedly inhibited pancreatic duct fluid and HCO_3^- secretion (458). Another important function of IRBIT is coordination of the ductal secretory process by regulating the activity of CFTR at the luminal membrane of the duct. Hence, IRBIT activates the native ductal CFTR and CFTR expressed in model system by direct interaction with CFTR to increase CFTR open probability (458). As was found with NBCe1-B, activation of CFTR by IRBIT is mediated by the PEST domain and is aided by assembly of IRBIT-CFTR complexes through their PDZ ligands (458). The findings in the native duct and with expressed NBCe1-B and CFTR are summarized in the model in Fig. 5, illustrating how IRBIT coordinates epithelial fluid and HCO_3^- secretion.

Activation of NBCe1-B and CFTR by IRBIT sets the ductal stimulated state of fluid and HCO_3^- secretion. This raised the question of how the duct resting state is set and whether IRBIT communicates with the regulators of the resting state. Inhibition of CFTR (455, 456) and Slc26a6 (193, 325) by the WNK kinases prompted examining the role of the WNK/SPAK kinases in the duct and their communication with IRBIT (456). As discussed above, the first 120 N terminal residues of the WNKs upstream of the kinase domain function as scaffolds (148, 155, 456) that recruit SPAK to NBCe1-B and CFTR. In turn, SPAK phosphorylates NBCe1-B and CFTR to cause their endocytosis and inhibit ductal fluid and HCO_3^- secretion (456). Significantly, IRBIT overrides the function of the WNK/SPAK pathway by recruiting the phosphatase PP1 to dephosphorylate NBCe1-B and CFTR, insert them into the basolateral and luminal membranes, respectively, and stimulate ductal fluid and HCO_3^- secretion (456) (Figure 5).

As indicated above, another component of the duct resting state is HCO_3^- salvage by the luminal NHE3 and NBCn1-A (233, 250). It is of note that other members of the Na^+-driven HCO_3^- transporters have an N terminal domain similar to that of NBCe1-B (35), including the electroneutral NBCn1 and the Na^+-dependent Cl^-/HCO_3^- exchangers NDCBE. It is thus possible that NBCn1, including the ductal NBCn1-A, are regulated by IRBIT, as was suggested (35). However, it is not clear which NBCn1 and NDCBE isoforms are activated

by IRBIT and whether IRBIT activate them by the same mechanism as NBCe1-B since the N terminal domain of NBCe1-A~C, NBCn1-A~H and NDCBE-A~D differ substantially. NHE3 was also reported to be modestly activated by IRBIT (149), which may be dependent on Ca^{2+} (150). Interestingly, NHE3 (3) and NBCn1 (327) are inhibited by CFTR at the luminal membrane. Hence, IRBIT appears to participate in the resting and stimulated state. It is possible that in the resting state part of IRBIT is sequestered by IP_3Rs and part of IRBIT is associated with NHE3 and NBCn1-A to promote HCO_3^- salvage. Once the cells are stimulated, IRBIT may dissociate from the HCO_3^- salvage, interact with the HCO_3^- secretory transporters and stimulate ductal fluid and HCO_3^- secretion. The model in Figure 5 illustrates the multiple regulatory roles of IRBIT.

D. Carbonic Anydrases

A class of proteins essential for all HCO_3^- related functions is the carbonic anhydrases (CAs). The global CA inhibitor acetazolamide significantly inhibits pancreatic (95, 321) and minimally inhibit salivary (461) fluid and HCO_3^- secretion. Further studies revealed that CAs are present at the HCO_3^- transporting complex, and supply HCO_3^- to several transporters. The CAs physically interact with and regulate the activity of several HCO_3^- transporters, including Na^+-HCO_3^- co-transporters and the AE and SLC26A6 Cl^-/HCO_3^- exchangers (269). Immunolocalization suggested localization of CAII, CAIV, CAIX, and CAXII in pancreatic (301) and multiple isoforms in salivary gland ducts (18, 172). Several of the ductal Ca^{2+} signal are cytoplasmic and some are integral membrane proteins with the catalytic domain in the cell surface. It is not clear at present which of these CAs is directly coupled to the basolateral NBCe1-B and AE2 in the ducts. Interestingly, the trafficking of CAIV to the luminal membrane is dependent on CFTR, and acetazolamide inhibits HCO_3^- transport by SLC26A3 (102) and SLC26A6 (269). These findings imply the involvement of the ductal CAs in HCO_3^- transport by the CFTR-SLC26Ts complex at the luminal membrane.

VII. DISEASES OF DUCTAL HCO_3^- SECRETION

Altered fluid and HCO_3^- secretion is associated with several diseases that affect various organs, although the pancreas is the most vulnerable to aberrant fluid and HCO_3^- secretion. The best known of these diseases is CF (94). It is obvious why a lack of CFTR function virtually eliminates ductal function. However, CF has a spectrum of phenotypes from mild to very severe depending on the mutation and sometime even within the same mutation (475), in particular in lung function (275). Interestingly, the CF genotype-phenotype correlates best with the state of pancreatic function (375, 378). Examining several mutations suggested that this relates to the ability of CFTR to support HCO_3^-, rather than Cl^-, transport (61), probably by stimulating the SLC26 transporters.

Another disease associated with aberrant HCO_3^- secretion is pancreatitis (70, 211, 409). Pancreatitis is an inflammatory disease that results in destruction of the pancreas by toxins that affect the acinar and duct cells (236). The most common form of pancreatitis is alcoholic pancreatitis, although many other causes of pancreatitis are known (138, 322), including autoimmune pancreatitis (211, 339). Most forms of pancreatitis are considered idiopathic. The central role of CFTR in ductal function led to a search of CFTR mutations associated with pancreatitis (63). The search identified several CFTR mutations that do not result in any of the CF symptoms, but are associated with chronic pancreatitis (62, 63, 230). Interestingly, recent analysis of two such mutations showed that they had no effect on Cl^- function of CFTR, but specifically reduced CFTR-mediated HCO_3^- transport (381, 436), further emphasizing the importance of HCO_3^- secretion in ductal function.

Of particular interest is a recent study that asked whether aberrant CFTR function is associated with idiopathic forms of chronic pancreatitis (211). Patients with autoimmune pancreatitis have reduced fluid and HCO_3^- secretion. Examination of CFTR localization in pancreatic biopsies showed mislocalization of CFTR and its accumulation in intracellular compartments (211). Most notably, treating the patients with corticosteroids corrected the mislocalization of CFTR to restored pancreatic HCO_3^- secretion and ameliorates disease severity (211). Moreover, preliminary results suggest that similar CFTR mislocalization occurs in alcoholic pancreatitis (211). It will be of particular interest to further analyze CFTR expression in all forms of pancreatitis, and if aberrant, whether correction of CFTR localization reduce disease symptoms. In addition, it will be informative to determine the fate of the SLC26 transporters in the disease, as they are tightly associated with CFTR physically and functionally (214, 215).

Another autoimmune inflammatory disease in which HCO_3^- secretion by salivary glands is aberrant is the dry-mouth, dry-eye disease Sjögren's syndrome (7). The cause of the aberrant HCO_3^- secretion is not known. However, one study reported that treating Sjögren's syndrome patients with the steroid prednisolone reduced damage and increased secretion by salivary glands (279). The results of treating autoimmune pancreatitis with corticosteroids (211) suggest that CFTR and perhaps the SLC26 transporters localization and/or function are altered in Sjögren's syndrome to reduce HCO_3^- secretion. Moreover, the reduced ductal fluid and HCO_3^- secretion in two autoimmune inflammatory diseases raise the possibility that many inflammatory diseases of secretory glands likely lead to some extent of reduced ducal HCO_3^- secretion.

HCO_3^- secretion is also reduced in patients undergoing radiation treatment for head and neck malignancies (7, 92). In this case it is likely that radiation resulted in ductal cell damage. Radiation damage is most commonly associated with severe oxidative damage (2, 473). Hence, it is possible that other forms of oxidative damage cause reduced ductal HCO_3^- secretion, in particular considering that CFTR activity is susceptible to oxidative damage (385). Indeed, in rat and mouse models of bile acid-induced pancreatitis oxidative stress causes marked reduction in ductal function that can be prevented by *in vivo* stimulation of signaling pathways that reduced oxidative stress (290, 292). Analysis of ductal transporters in these diseases should reveal the cause of the disease, suggest treatment modalities, and further clarify the mechanism and regulation of ductal fluid and HCO_3^- secretion.

VIII. MECHANISM AND A MODEL FOR DUCTAL HCO_3^- SECRETION

A. Basic Concepts

A unique feature of the duct in most species, including humans, is the secretion of copious amount of HCO_3^- with the pancreatic duct secreting fluid containing as much as 140 mM HCO_3^- (237). Accordingly, the mechanism and regulation of epithelial HCO_3^- secretion is understood best in the pancreatic duct and thus the model we discuss reflects best the mechanism of fluid and HCO_3^- secretion by the pancreatic duct. However, although not understood at the same detail, the available data indicates that the mechanism discussed below for the pancreatic duct is applicable to other exocrine gland ducts with variations that meet specific glands specialization, but do not deviate from the basic principles, as we tried to emphasis with the salivary gland ducts example.

The duct secretes most of the fluid in the pancreatic juice and while doing so it absorbs the Cl^- and secretes HCO_3^- to generate pancreatic juice containing about 20 mM Cl^- and 140 mM HCO_3^-. This task imposes several criteria for the mechanism of pancreatic duct fluid and HCO_3^- secretion that must be met. First, the basolateral HCO_3^- influx mechanism must

be able to concentrate HCO_3^- in the duct cytosol. Second, HCO_3^- efflux mechanism at the luminal membrane must be able to concentrate HCO_3^- at the luminal space and the luminal membrane must have low HCO_3^- leak and maintain a membrane potential with negative transcellular potential. While absorbing Cl^- and secreting HCO_3^-, the pancreatic duct must mediate large net transcellular salt transport necessary for copious fluid secretion by the duct. Since the only osmolyte actively secreted by the pancreatic duct is HCO_3^-, the bulk of HCO_3^- secretion cannot be by osmotically silent HCO_3^- transport mechanism, such as coupled electroneutral $1Cl^-/1HCO_3^-$ exchange or uncoupled $1Cl^-/1HCO_3^-$ exchange by a Cl^- and HCO_3^- permeable channels, such as CFTR, or Cl^- and K^+ cotransport by luminal Cl^- and K^+ channels. These set specific constrains on the basolateral HCO_3^- influx mechanism and the luminal Cl^- absorbing and HCO_3^- secreting mechanism. The capacity of the basolateral HCO_3^- absorbing mechanism must exceed the capacity of basolateral HCO_3^- efflux mechanisms, such as AE2, and the luminal HCO_3^- exit mechanism must function with a stoichiometry of $HCO_3^-/Cl^- > 1$.

The first attempt to explain pancreatic duct fluid and HCO_3^- secretion was made in the late 1980's by Argent and Case (14). The basic tenants of their model are the basolateral Na^+/K^+ pump and K^+ channels, which generate the Na^+ and K^+ gradients and set the membrane potential that are needed to fuel the secretion. A HCO_3^- entry equivalent is provided by NHE. HCO_3^- then exits the luminal membrane by way of a Cl^-/HCO_3^- exchange that absorbs the Cl^-, with the Cl^- re-circulates by exiting through CFTR (14, 404). Although the model does not meet the constraints listed above and thus cannot explain ductal fluid and HCO_3^- secretion, it was an important model in drawing attention to the problem and stimulating the study of ductal secretion.

Several key discoveries led to significant revision of this model. The major basolateral HCO_3^- influx mechanism was identified as NBCe1-B, which functions as a $1Na^+$-$2HCO_3^-$ cotransporter (1, 180, 471). The main luminal Cl^-/HCO_3^- exchanger in the pancreatic and salivary ducts was identified as Slc26a6 (215, 247), which functions as electrogenic, coupled $1Cl^-/2HCO_3^-$ exchanger (313, 388) that interacts with CFTR, with Slc26a6 and CFTR activating each other (215). Modeling and studies in guinea pig pancreatic ducts suggested a requirement for HCO_3^- channel activity to set the final pancreatic juice HCO_3^- at 140 mM (400, 401, 437). These findings led a two stage model in which initially HCO_3^- is mediated by electroneutral Cl^-/HCO_3^- exchange. Higher HCO_3^- concentration in the pancreatic juice is achieved by CFTR acting as a HCO_3^- channel (for review see (237, 404)). A significant problem with the model is that it does not result in osmotic secretion of HCO_3^- and thus will not lead to fluid secretion. In addition, the model does not explain how CFTR can conduct HCO_3^- at the terminal portion of the duct.

B. A Model of Ductal Fluid and HCO_3^- Secretion

The following findings were used to develop the model in Fig. 6. Loss of pancreatic HCO_3^- secretion in patients with CF (191) indicates that CFTR plays a critical role in HCO_3^- secretion. The activity of the duct apical Cl^-/HCO_3^- exchanger is inhibited by DIDS (235, 405) (which does not inhibit CFTR) and is dependent on the expression of CFTR (235, 238). CFTR mutations associated with pancreatic insufficiency exhibit a severe defect in CFTR-dependent Cl^-/HCO_3^- exchange activity (61). Coupled or uncoupled, channel-mediated 1:1 Cl^-/HCO_3^- exchange cannot account for either HCO_3^- or fluid secretion by the duct (154). HCO_3^- secretion must be an osmotically active process. Electrogenic HCO_3^- transporters can generate a fluid with high HCO_3^- concentrations using the driving force generated by the negative luminal membrane potential. Apical Cl^-/HCO_3^- exchanger by the duct is electrogenic with distinct Cl^-:HCO_3^- stoichiometry (215). The duct expresses Slc26a6 (214, 215) that functions as a $1Cl^-/2HCO_3^-$ exchanger (313, 388). The WNK/SPAK pathway stabilizes the resting state in the proximal portion of the duct (456), while regulating CFTR

HCO_3^- selectivity and permeability in the distal portion of the duct, switching CFTR into a HCO_3^--selective channel (325). IRBIT governs ductal fluid and HCO_3^- secretion and overrides the inhibitory action of the WNK/SPAK pathway (456). The findings lead to a two stage model: In stage one the proximal duct secretes the bulk of fluid and HCO_3^- secretion that is mediated by Slc26a6 with CFTR recycling the Cl^-, the WNK/SPAK pathway regulating the resting state and IRBIT set the stimulated state. In step two, the WNK/SPAK pathway switches CFTR to largely a HCO_3^- channel to secret HCO_3^- and set the final HCO_3^- secretion of the pancreatic juice (Fig. 6).

With the steep inward Na^+ gradient of about 15 fold, $1Na^+$-$2HCO_3^-$ co-transport by NBCe1-B leads to active accumulation of cytosolic HCO_3^-. Secretion of HCO_3^- by the $1Cl^-$/$2HCO_3^-$ exchanger Slc26a6 leads to osmotic secretion of HCO_3^-. The obligatory movement of Na^+ through the paracellular pathway results in net Na^+-HCO_3^- secretion that drives water efflux by AQP1 and starts the process of accumulation of HCO_3^- in the duct lumen. In the resting state, the WNK/SPAK pathway keeps large fraction of NBCe1-B and of CFTR in intracellular compartments while IRBIT may be associated with and activate NHE3 and NBCn1-A (not included in the model) to salvage any Na^+ and HCO_3^- leaking to the duct lumen. Initiation of ductal secretion requires antagonism of the WNK/SPAK pathway by IRBIT-mediated recruitment of PP1 to dephosphorylate the transporters NBCe1-B and CFTR and exocytose them to their respective plasma membrane. Once at the plasma membrane the transporters are phosphorylated by PKA and now IRBIT interacts directly with the inhibitory domains in NBCe1-B, CFTR and perhaps Slc26a6 to activate them and set the stimulated state. At a luminal membrane potential of −50 mV or more negative, and the ionic gradients of HCO_3^- and Cl^- set by NBCe1-B and CFTR, $1Cl^-$: $2HCO_3^-$ exchange by Slc26a6 can achieve a luminal HCO_3^- concentration of about 136 mM (404).

To transport events in the proximal duct outlined above can account for the bulk of fluid and HCO_3^- secretion by the rodent duct. However, the guinea pig, pig and human pancreatic ducts can generate pancreatic juice containing more than 120–130 mM HCO_3^-. As luminal Cl^- is absorbed, the reduced Cl^- gradient across the luminal membrane limits HCO_3^- secretion by Slc26a6, and thus alternative pathway if HCO_3^- efflux is required to maintain HCO_3^- secretion. The decrease in luminal Cl^- and the activated CFTR at a membrane potential of −50 mV or more negative reduces $[Cl^-]_i$ to below 10 mM, which serves as an alternative signal to modulate the activity of WNK/SPAK pathway. Activation of the WNK/SPAK pathway by low $[Cl^-]_i$ converts CFTR from a Cl^- channel to preferably HCO_3^- channel, allowing further HCO_3^- efflux. The WNK/SPAK pathway inhibits any luminal Cl^-/HCO_3^- exchange activity to avoid HCO_3^- absorption due to the steep HCO_3^- gradient set by CFTR (325). IRBIT activity in this portion of the duct needs to be reduced (not included in the model), to allow the WNK/SPAK pathway to act on CFTR and the SLC26 transporters.

The model in Fig. 6 can explains the available information on the mechanism of fluid and HCO_3^- secretion by secretory gland ducts, most importantly how the duct can secrete both large fluid volume and high amount of HCO_3^-. By no means, this intent to be the last word in the mechanism by which secretory gland ducts secrete fluid and electrolytes. As with other models, including our earlier models, it will continue to evolve as knowledge of existing pathway increases and new pathways are discovered. However, we hope that at this stage the model will stimulate further research into this so very important topic of fluid and HCO_3^- secretion that is aberrant in large number of epithelial diseases.

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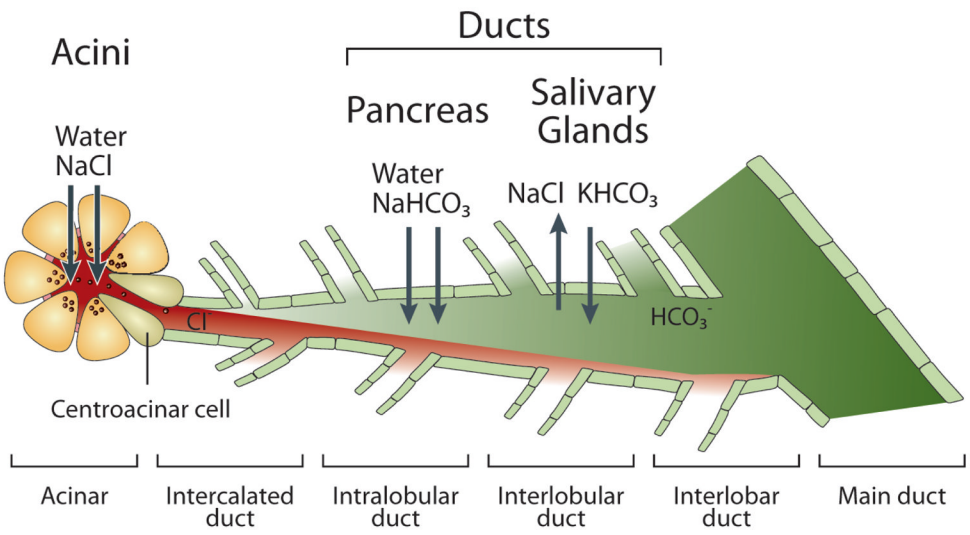

Fig. 1. The acinar and ductal segments of secretory glands and fluid and electrolyte secretory functions

The figure illustrates the relationships between the acinar and ductal portion of secretory glands. The acini secrete isotonic fluid with NaCl as the major salt. The fluid passes through the centroacinar cells to the duct. The pancreatic duct absorbs the Cl^- and secretes HCO_3^- and most of the water in the pancreatic juice. Active HCO_3^- secretion drives fluid secretion. The salivary duct absorbs both the Cl^- and the Na^+ and secretes HCO_3^- and K^+. ENaC is expressed in the salivary glands, but not in the pancreatic duct, and is the main pathway for Na^+ absorption by the salivary glands duct.

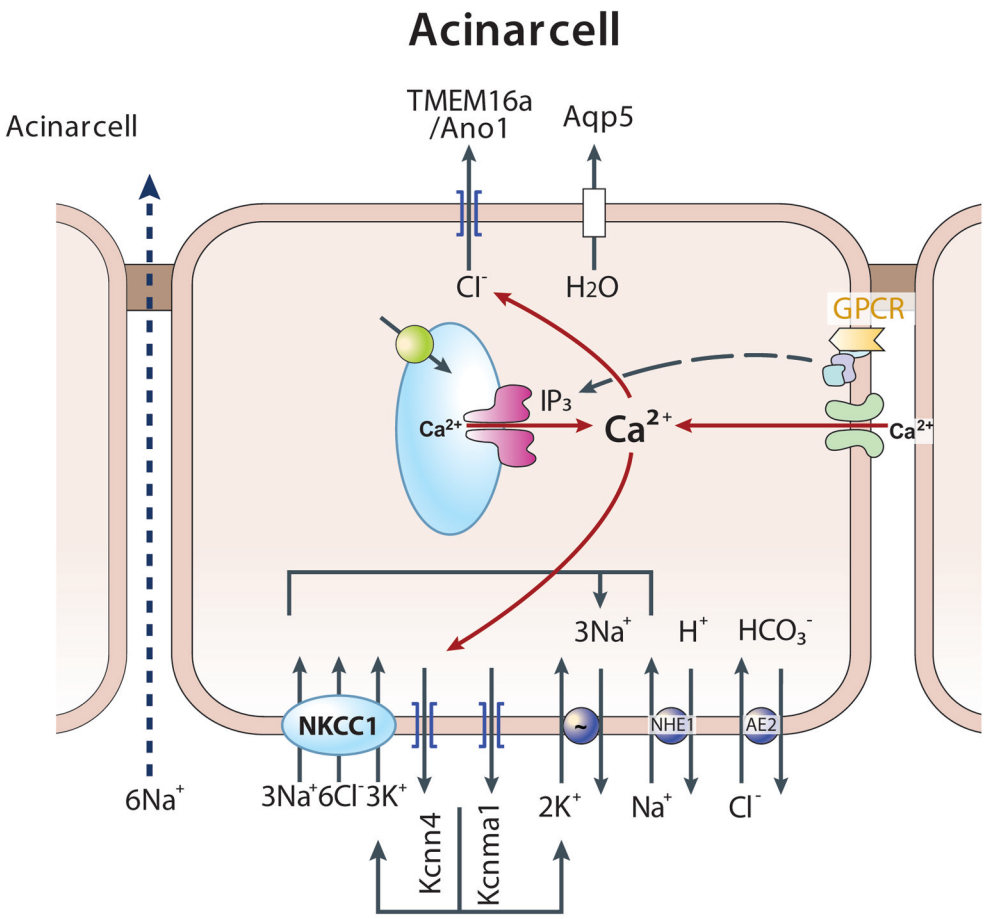

Fig. 2. A model depicting the mechanism of acinar cells fluid and electrolyte secretion

Shown are the major transporters in the basolateral and luminal membranes of acinar cells and their regulation. The major Cl^- loading transporter at the basolateral membrane is NKCC1, with part of the Cl^- loading (about 30%) provided by the parallel functioning of NHE1 and AE2. The membrane potential is determined by two Ca^{2+}-activated K^+ channels, the MaxiK and mIK1 channels. TMAM16a/Ano1 is the major Ca^{2+}-activated Cl^- channel at the luminal membrane that also expresses the water channel AQP5. Fluid and electrolyte secretion by acinar cells is regulated by Ca^{2+}-mobilizing receptors and is a Cl^- secretion-driven process. The receptor-evoked $[Ca^{2+}]_i$ increase initiates at the apical pole where the Ca^{2+} signaling complexes are located to activate TMEM16a/Ano1. The Ca^{2+} signal then propagates to the basal pole to activate the K^+ channels. The Ca^{2+}-mediated channels activation results in luminal Cl^- efflux and basolateral K^+ efflux. Na^+ then flows through the tight junction to the luminal space. The secretion of NaCl leads to water efflux through AQP5 and cell shrinkage. Cell shrinkage reduces $[Ca^{2+}]_i$ to inhibit the Cl^- and K^+ channels and at the same time activates the volume-sensitive NKCC1 (and NHE1 and AE2) to restore cell Cl^- and K^+. The cycle repeats itself during each spike of Ca^{2+} oscillations.

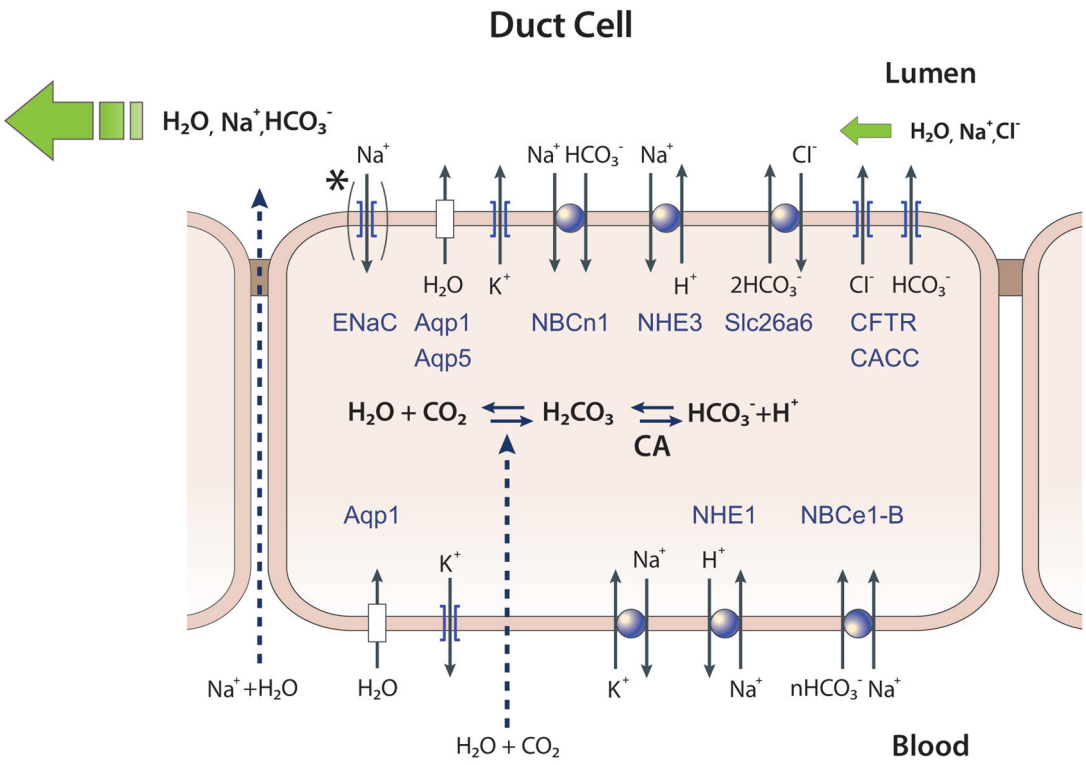

Fig. 3. A model depicting the mechanism of ductal fluid and HCO_3^- secretion

Shown are the major transporters in the basolateral and luminal membranes of duct cells. In the pancreas ductal secretion is driven by HCO_3^- secretion. The major HCO_3^- loading mechanism is the basolateral Na^+-HCO_3^- cotransporter NBCe1-B. Luminal HCO_3^- secretion is mediated by the CFTR-Slc26a6 complex. The duct also expresses the HCO_3^- salvage mechanisms NHE3 and NBCn1-A. The salivary glands, but not the pancreatic, duct also expresses ENaC at the luminal membrane. The functioning of the transporters in ductal fluid and HCO_3^- secretion is illustrated in Fig. 6.

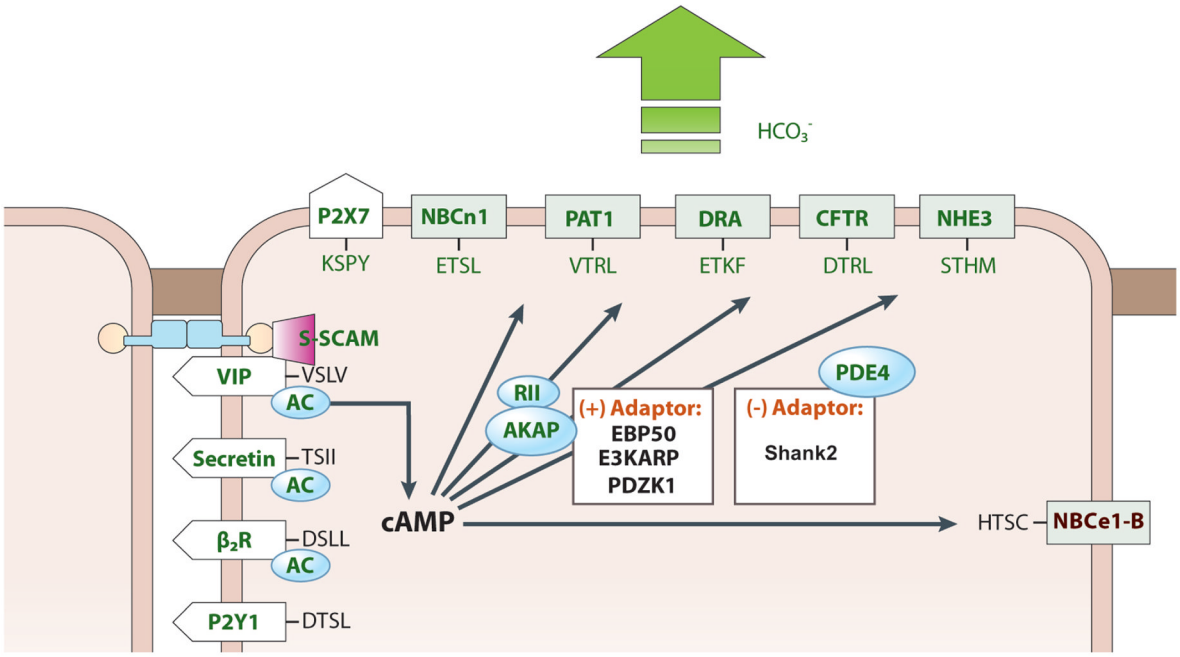

Fig. 4. Ductal proteins with PDZ ligands that participate in fluid and HCO_3^- secretion

All receptors that stimulate ductal secretion and key transporters that mediate ductal fluid and HCO_3^- secretion have PDZ ligands, highlighting the key role of PDZ scaffolds in ductal function. See text for details.

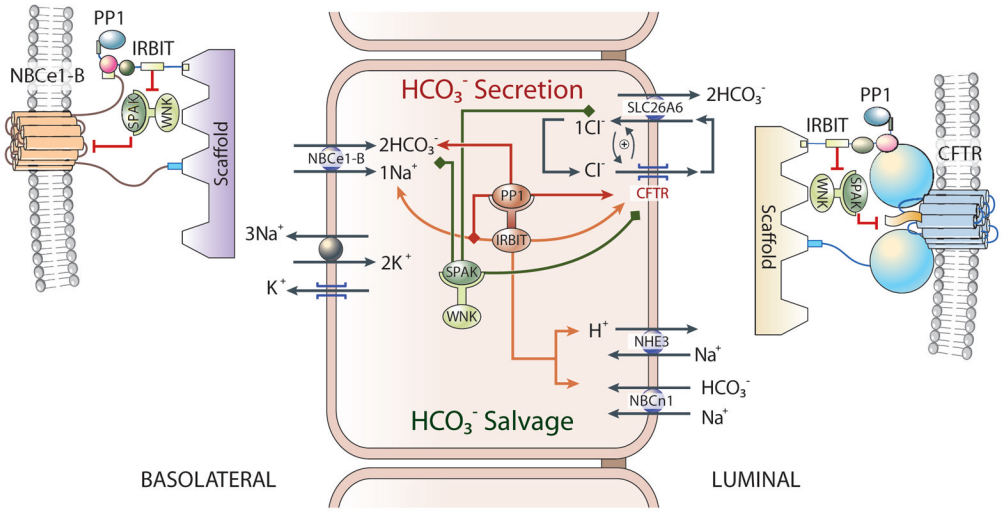

Fig. 5. The IRBIT/PP1 and WNK/SPAK pathways in ductal function

IRBIT is a key regulator of ductal fluid and HCO_3^- secretion that regulates both the resting and stimulated states of ductal secretion. PZD scaffolds assemble a basolateral membrane complex composed of NBCe1-B, the WNK/SPAK kinases and IRBIT (which can recruit the phosphatase PP1 to the complex). Similar complex exists in the luminal membrane with CFTR and perhaps Slc26a6 as the major transporters. In the resting state the WNK/SPAK kinases phosphorylate all transporters to reduce their surface expression and thus activity. Part of IRBIT is sequestered by IP_3Rs and part is bound to NHE3 and NBCn1-A to activate them and thus affect HCO_3^- salvage. Upon cell stimulation IRBIT recruits PP1 to the complexes, which overrides the function of the WNK/SPAK pathway and dephosphorylates the HCO_3^- secreting transporters to increase their surface expression. IRBIT then binds and neutralizes the effect of the HCO_3^- secreting transporters inhibitory domains. The combined effects stabilize the secretory state of the duct. The mutual stimulation of CFTR and Slc26a6 in the complex further augments ductal secretion.

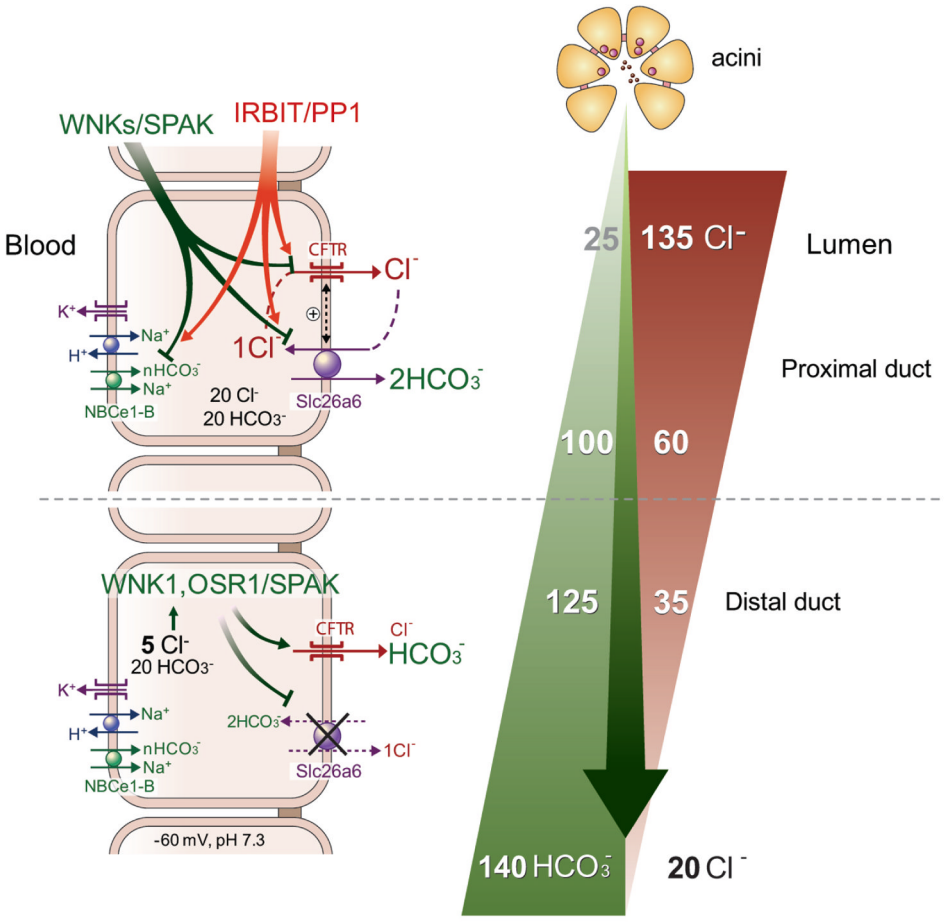

Fig. 6. A model of pancreatic duct fluid and HCO_3^- secretion

Ductal fluid and HCO_3^- secretion is a two stage process. In the proximal duct IRBIT antagonizes the effect of the WNK/SPAK pathway to stimulate ductal secretion. HCO_3^- accumulates in the duct cytosol by NBCe1-B and exits into the lumen mostly by Slc26a6, which mediates $1Cl^-/2HCO_3^-$ exchange, with CFTR recycling the Cl^-. This result with osmotic secretion of HCO_3^- and together with transcellular Na^+ fluxes through the paracellular pathway drives fluid secretion. The water is secreted by AQP1. The proximal duct thus absorbs part of the Cl^- and secretes as much as 100 mM HCO_3^- to secret large fraction of the fluid in the pancreatic juice. As the fluid arrives the more distal portions of the duct, the reduced luminal Cl^- and activated CFTR results in intracellular Cl^- concentration ($[Cl^-]_i$) of less than 10 mM. The low $[Cl^-]_i$ activates WNK1 that phosphorylates SPAK/OSP1, which, in turn, acts on CFTR to change its Cl^-/HCO_3^- selectivity, converting it primarily a HCO_3^- channel. At the same time the WNK/SPAK pathway inhibits the function of Slc26a6 to prevent HCO_3^- re-absorption. HCO_3^- efflux by CFTR thus determines the final HCO_3^- concentration in the secreted fluid.

Table 1

Transporters in the luminal (apical) membrane of exocrine gland ducts

Transporter	Protein	Role	References
cAMP-activated Cl^- channel	CFTR (ABCC7)	Fluid and HCO_3^- secretion	(126), (127), (125), (128), (4), (312), (296), (26), (261), (68), (466), (183), (103), (175), (174), (245), (340), (353), (387), (182), (84), (324), (402), (61)
Ca^{2+}-activated Cl^- channel	TMEM16/Anotcamin family (?)	(?)	(128), (444), (108), (443), (105), (296), (4), (158), (21), (176), (39), (452), (51), (52), (55), (345), (476), (459), (43), (383)
Anion exchangers		HCO_3^- secretion	(47), (220), (19), (407), (306), (471), (235), (153), (20), (97), (291), (238), (213), (386), (401), (183), (179), (178)
	SLC26A3 (DRA/CLD)	HCO_3^- secretion, electrogenic $2Cl^-/1HCO_3^-$ exchanger	(96), (273), (129)
	SLC26A6 (PAT1)	Fluid and HCO_3^- secretion, electrogenic $1Cl^-/2HCO_3^-$ exchanger	(96, 247), (429), (209), (190), (434), (449), (213), (235), (215), (122)
Na^+/H^+ exchangers	NHE3 (SLC9A3)	HCO_3^- reabsorption (HCO_3^- salvage)	(118), (263), (471), (264), (234)
	NHE2 (SLC9A2)	(?)	(118), (263), (471), (264), (234)
Na^+-HCO_3^- cotransporter	NBCn1-A (NBC3, SLC4A7)	HCO_3^- reabsorption (HCO_3^- salvage) ?	(327), (59), (250)
K^+ channels	Maxi- K^+ channels (KCNMA1?)	Maintain membrane potential during stimulated secretion	(423)
Water channel	Aquaporin 5 (AQP5)	Fluid secretion	(42)
Epithelial Na^+ channel (ENaC)	SCNN1A (α), SCNN1B (β), SCNN1G (γ), SCNN1D (δ)	Na^+ absorption (Functional ENaC channel with αβγ subunits is expressed in salivary, but not in pancreatic duct. ENaCδ is expressed in pancreas; however its role is uncertain.)	(49, 65, 454)

Table 2

Transporters in the basolateral membrane of the ducts

Transporter	Protein	Role	References
Na^+/H^+ exchangers	NHE1 (SLC9A1)	Na^+/H^+ exchange, Contributes to basolateral HCO_3^- influx	(407), (305), (471), (180), (72), (178), (234), (370),
	NHE4 (SLC9A4)	(?)	(370), (234)
H^+-ATPase	V-type H^+- ATPase	(?)	(349), (40), (421), (420), (428), (300), (471), (370), (72), (183), (179), (133), (54)
Na^+-HCO_3^- cotransporters	NBCe1-B (pNBC1, SLC4A4)	The major Basolateral HCO_3^- uptake transporter	(221), (471), (428), (180), (72, 178), (396), (182), (1), (413), (260), (379), (371), (131), (131), (132, 179), (173)
Anion exchangers	AE2 (SLC4A2)	Housekeeping function Prevent intracellular alkalinization	(471), (235), (178), (177), (171), (129), (370), (19)
Cation-chloride cotransporters	Na^+-K^+-2Cl^- cotransporter (NKCC1, SLC12A2)	Basolateral Cl^- uptake in mouse and rat ducts, but not in guinea pig and human)	(103), (54), (397), (67)
	K^+-Cl^- cotransporter (KCC1, SLC12A4)	Basolateral $K^+$and Cl^- efflux Cell volume regulation?	(119), (373)
K^+ channels	Maxi- K^+ channels (KCNMA1)	Maintain membrane potential	(305), (157), (308), (320), (168), (152), (124), (296), (15), (19, 123)
	Small or intermediate conductance K^+ channels (mIK1)	Maintain resting membrane potential	(305, 404)
Na^+,K^+-ATPase	Na^+,K^+- ATPase (ATP1B1)	Maintain inward Na^+ and outward K^+ gradients	(305), (256), (41), (399)
Water channels	Aquaporin 1 (AQP1)	Water transport.	(109), (212), (42)
	Aquaporin 5 (AQP5)	Water transport. Role in secretion unknown at present	(42)