

The Physiology and Pathophysiology of Pancreatic Ductal Secretion

The Background for Clinicians

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Abstract: The human exocrine pancreas consists of 2 main cell types: acinar and ductal cells. These exocrine cells interact closely to contribute to the secretion of pancreatic juice. The most important ion in terms of the pancreatic ductal secretion is HCO_3^- . In fact, duct cells produce an alkaline fluid that may contain up to 140 mM NaHCO_3 , which is essential for normal digestion. This article provides an overview of the basics of pancreatic ductal physiology and pathophysiology. In the first part of the article, we discuss the ductal electrolyte and fluid transporters and their regulation. The central role of cystic fibrosis transmembrane conductance regulator (CFTR) is highlighted, which is much more than just a Cl^- channel. We also review the role of pancreatic ducts in severe debilitating diseases such as cystic fibrosis (caused by various genetic defects of *cftr*), pancreatitis, and diabetes mellitus. Stimulation of ductal secretion in cystic fibrosis and pancreatitis may have beneficial effects in their treatment.

Key Words: pancreas, ductal secretion, cystic fibrosis, CFTR, pancreatitis, diabetes mellitus

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The human exocrine pancreas consists of 2 main cell types: acinar and ductal cells. These exocrine cells interact closely to contribute to the secretion of pancreatic juice.¹ Acinar cells (which make up >80% of the pancreatic mass) secrete an isotonic, NaCl - and H^+ -rich fluid containing various digestive enzymes.² The secreted Cl^- is then exchanged to HCO_3^- by duct cells to produce an alkaline fluid that may contain up to 140 mM NaHCO_3 , which is essential for normal digestion.^{3–5} Although volume-wise the ducts cells account for approximately only 5% of the pancreas, a large proportion of the secreted pancreatic fluid is due to the duct cells. Under stimulated conditions, duct cells secrete a large quantity of electrolytes, which is followed by fluid movement. Ductal HCO_3^- concentration in guinea pig (which is a commonly used model animal to study pancreatic secretion) can be as high as in humans; however, rats or mice can secrete only 70 to 80 mM HCO_3^- .^{5,6} Although the exact mechanism of ductal HCO_3^- and fluid secretion is only partially understood, it is evident that the differences in HCO_3^- concentration of the various species are due to the different expression of apical

and basolateral transporters involved in the secretory process. In all cases, the physiological function of this alkaline fluid is to neutralize the acidic content secreted by acinar cells, to provide an optimal pH for digestive enzymes, to flush down digestive enzyme into the duodenum, and also to neutralize the gastric acid entering the duodenum.⁷ Importantly, HCO_3^- has a crucial biochemical role in the physiological pH buffering system and is a chaotropic agent that prevents the denaturing of proteins such as digestive enzymes and mucins so it facilitates their solubilization in biological fluid.^{4,8}

Investigating the mechanisms of pancreatic ductal HCO_3^- and fluid secretion also helps us to better understand pancreatic diseases.⁴ Impaired ductal secretion can result in pancreatic damage, as seen in cystic fibrosis (CF),^{3,9} and may contribute to the development of other diseases such as acute and chronic pancreatitis.¹⁰

The aim of this review is to summarize the physiology and pathophysiology of pancreatic ductal epithelial cells (PDECs). We will try to keep things simple and not go into too much molecular detail. These have been discussed in recent reviews by distinguished experts in the field such as Argent et al,¹¹ Ishiguro et al,³ Lee et al,⁶ group of Muallem,¹² and Novak et al.¹³ With respect to ductal pathophysiology, only CF, pancreatitis, and diabetes mellitus are discussed, and we will not deal with pancreatic adenocarcinoma (mainly arising from ductal cells).

MECHANISM OF PANCREATIC DUCTAL SECRETION

For a long time, it was believed that the main function of PDECs is to ensure mechanical frame for acinar cells. In 1986, Barry Argent and his colleagues¹⁴ have worked out a method that made it possible to isolate intact pancreatic ducts and PDECs. This was a landmark discovery, because until then, ductal function could be investigated only in intact animals. From then on, it was possible to separately study the function of duct cells, and numerous publications proved that PDECs are responsible not only for the formation of a mechanical frame for the acini, but also for the HCO_3^- and fluid secretion of the pancreatic juice.³ The development of pancreatic ductal cell lines have also helped us in understanding the secretory process, but as these are mainly derived from adenocarcinomas, their function may be compromised.

Whereas acinar cells have a relatively uniform morphology, the structure of duct cells is much more diverse. Perhaps the most enigmatic cell type of the exocrine pancreas is the centroacinar cells, which are localized at the junction of the acini and are closely associated with the terminal ductal epithelium.¹⁵ Epithelia are cuboidal along the proximal small ducts and are columnar in the distal large ducts.¹⁶ Therefore, it is not surprising that proximal and distal duct cells also differ in their function. HCO_3^- secretion is thought to occur primarily in the proximal part of the ducts.³

The Model of Pancreatic Ductal HCO_3^- Secretion

The exact mechanism how the exocrine pancreas secretes a large amount of the alkaline fluid has long been an enigma. Note

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that the HCO_3^- concentration of the pancreatic juice in the stimulated state is more than 5 times that found in the serum. Major milestones in understanding the mechanism of pancreatic HCO_3^- secretion include the discovery of the acidic pancreatic juice in patients with CF,¹⁷ the isolation of pancreatic ducts,¹⁴ and the molecular identification of several ion channels and transporters of PDECs, such as the CF transmembrane conductance regulator (CFTR),¹⁸ the $\text{Na}^+/\text{HCO}_3^-$ cotransporter (NBCe1-B, also known as pNBC1),¹⁹ and the solute carrier family 26 (SLC26) transporters.^{20,21} Our knowledge has also expanded about how ductal secretion is regulated.⁶

Pancreatic ductal HCO_3^- secretion is a complex process that can be broadly divided into 2 separate steps. The first step of HCO_3^- secretion is the accumulation of HCO_3^- inside the duct cell across the basolateral membrane. This can be achieved via a direct mechanism through $\text{Na}^+/\text{HCO}_3^-$ cotransporters or indirectly via the passive diffusion of CO_2 through the cell membrane and the conversion of CO_2 to HCO_3^- and H^+ mediated by carbonic anhydrase²² and backward transport of protons by Na^+/H^+ exchangers (NHEs) and an H^+ -ATPase.³ The second step of HCO_3^- secretion across the apical membrane of PDECs is thought to be mediated by anion channels and transporters such as CFTR and SLC26 anion exchangers³ (Fig. 1).

How these transporters act in concert to produce a high HCO_3^- secretion in humans is controversial. One hypothesis is that HCO_3^- is secreted via the electroneutral $\text{Cl}^-/\text{HCO}_3^-$ exchanger until the luminal concentration reaches about 70 mM, after which the additional HCO_3^- required to raise the luminal concentration to 140 mM is transported by CFTR.⁵ Another hypothesis suggests that 2 electrogenic SLC26 anion exchangers with isoform-specific stoichiometry mediate HCO_3^- secretion at different sites along the ductal tree, and CFTR functions to activate the exchangers and to provide the luminal Cl^- required for anion exchange to occur.^{4,6,23}

Electrolyte and Fluid Transporters of Pancreatic Ductal Cells

Cystic Fibrosis Transmembrane Conductance Regulator

Cystic fibrosis transmembrane conductance regulator, the most critical player in HCO_3^- secretion, was discovered 25 years ago as the gene whose mutation is responsible for CF.^{18,24,25} It is a cAMP-activated Cl^- channel found in the plasma membrane, is a member of the ATP-binding cassette transporter superfamily. ATP-binding cassette transporters utilize the energy of ATP binding and hydrolysis to carry out certain biological processes.²⁶ In fact, ATP-binding cassette transporters have 2 distinct domains:

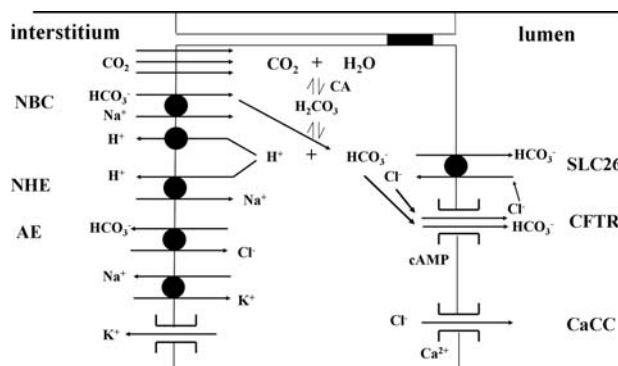


FIGURE 1. Schematic diagram of ion transport systems in pancreatic ductal epithelial cells. AE, anion exchanger.

nucleotide-binding domains and transmembrane domains, which contain several membrane-spanning α -helices and a regulatory (R) domain that is phosphorylated by protein kinase A and protein kinase C (PKC).^{27,28} In addition, CFTR contains several other domains mediating protein-protein interactions, including postsynaptic density 95/disc-large/zonula occludens 1 (PDZ)-interacting domains in the C terminus. Proteins that contain PDZ domains often have other protein-interacting modules (such as ezrin, radixin, moesin-binding domains, and coiled-coil domains) and therefore can promote homotypic and heterotypic protein-protein interactions.²⁹

Cystic fibrosis transmembrane conductance regulator is found in the epithelial cells of many organs including the pancreas, lung, liver, digestive tract, reproductive tract, and skin. Although CFTR is predominantly a Cl^- channel, it can also conduct other anions. Gray et al³⁰ provided clear evidence that CFTR can transport HCO_3^- in pancreatic duct cells, but CFTR is 3 to 5 times more selective for Cl^- over HCO_3^- .³¹ Interestingly, $\text{Cl}^-/\text{HCO}_3^-$ selectivity of CFTR is dynamic and is regulated by external Cl^- .³² According to these studies, when Cl^- is present in physiologic concentration in the lumen of proximal pancreatic ducts, CFTR functions as a Cl^- channel and does not carry HCO_3^- . However, when luminal $[\text{Cl}^-]$ and $[\text{Cl}^-]_i$ are low at the distal part of pancreatic ducts, CFTR secretes HCO_3^- across the apical membrane of the ductal cells.^{33,34} It has been shown that CFTR Cl^- currents were rapidly inhibited by HCO_3^- in a voltage-independent manner.³⁵ Cystic fibrosis transmembrane conductance regulator Cl^- permeability is switched by the With-No-Lysine (WNK)/STE20/SPS1-related proline/alanine-rich kinase (SPAK) kinase pathway (which is regulated by $[\text{Cl}^-]_i$), making CFTR an HCO_3^- -permeable channel.^{34,36} Inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R)-binding protein released with IP_3 (IRBIT), which is another recently described regulatory protein, also appears to play a fundamental role in the regulation of HCO_3^- secretion. In addition, IRBIT seems to mediate synergism between intracellular Ca^{2+} and cAMP signaling.³⁷

Another observation that highlighted the crucial role of CFTR in pancreatic HCO_3^- secretion is that CFTR mutations associated with exocrine pancreatic insufficiency also show a major deficiency in the apical CFTR-dependent $\text{Cl}^-/\text{HCO}_3^-$ exchange activity.^{38,39} In addition to acting as a Cl^- channel, CFTR also directly or indirectly regulates several transport proteins via formation of macromolecular complexes. Functional interactions with CFTR were reported for the epithelial Na^+ channel, K^+ channels, SLC26 anion exchangers, Ca^{2+} activated Cl^- channel, $\text{Na}^+/\text{HCO}_3^-$ transporters (NBCn1-A), NHEs, and aquaporin (AQP) water channels.^{4,28,40,41} Ko et al⁴² provided important evidence for the functional interaction between CFTR and select SLC26 transporters (SLC26A3, SLC26A4, and SLC26A6) and later localized the relevant interacting regions to the R domain of CFTR and the carboxyl terminus (sulfate transporter and anti-sigma factor antagonist [STAS] domain) of SLC26 transporters.²³ The interactions of CFTR and other transporters are mediated by protein-binding domains. In fact, the PDZ-interacting domain of human CFTR mediates its binding to several PDZ domain-containing proteins, including NHE regulatory factor isoform 1-4 (NHERF-1 to NHERF-4) and CFTR-associated ligands.⁴³⁻⁴⁵ It has also been demonstrated that the regulation of transporters by CFTR not only goes one way, but SLC26A6 can also modify CFTR activity in both the resting and stimulated states.⁴⁶

$\text{Cl}^-/\text{HCO}_3^-$ Exchangers: Solute Carrier Families 4 and 26

$\text{Cl}^-/\text{HCO}_3^-$ exchangers are encoded by the SLC4 and SLC26 gene superfamilies and function to regulate intracellular pH,

[Cl⁻], and cell volume.⁴⁷ The SLC4 family includes 4 distinct Na-independent Cl⁻/HCO₃⁻ exchangers known as AE1, AE2, AE3, and AE4, with AE1-3 exclusively located on the basolateral membrane of epithelial cells.⁴⁸ Pancreatic duct cells express the housekeeping AE2 exchanger (SLC4A2)^{49,50} that mediates electroneutral exchange of 1 Cl⁻ (into the cell) and 1 HCO₃⁻ (from the cell to the interstitium).⁵¹ The activity of the latter transporter is likely to be inhibited during stimulated secretion (as it acts against it), which has been confirmed in guinea pig.⁵¹ The discovery of the SLC26 family of luminal Cl⁻/HCO₃⁻ exchangers was a breakthrough in understanding the mechanism of pancreatic HCO₃⁻ secretion.⁵² SLC26 isoforms are large, structurally well-conserved anion exchangers with highly restricted and distinct tissue distribution. The C-terminal cytoplasmic region of all SLC26 proteins includes a “sulfate transporter and anti-sigma factor antagonist (STAS) domain,” which contains PDZ recognition motifs.⁵³ To date, 10 SLC26 genes or isoforms (SLC26A1-SLC26A11) have been cloned (SLC26A10 is a pseudogene).⁴⁸ The family members have diverse substrate specificity. SLC26A1 and SLC26A2 were identified as SO₄²⁻ transporters,²¹ SLC26A3 and SLC26A6 function as Cl⁻/HCO₃⁻ exchangers,^{42,54} SLC26A4 is an electroneutral Cl⁻/HCO₃⁻/I⁻ exchanger,⁵⁵ SLC26A5 functions as an anion regulated, voltage-dependent motor protein.⁵⁶ SLC26A7,⁵⁷ SLC26A9,⁵⁸ and SLC26A11⁵⁹ are Cl⁻ channels.⁶ The function of SLC26A8 is unclear, but it exhibits modest transport of Cl⁻, SO₄²⁻, and oxalate.⁶⁰

In pancreatic ducts, the expression of SLC26A2,⁶¹ SLC26A3,⁶² SLC26A6,^{50,62,63} and SLC26A11⁶⁴ was detected. SLC26A2 immunoreactivity was localized to the epithelia of large pancreatic ducts in humans; however, no functional data are available on its activity. SLC26A3 and SLC26A6 were localized to the apical membrane of human PDECs^{62,63} and are thought to have important roles in the mechanism of pancreatic ductal HCO₃⁻ secretion.^{23,48,50} SLC26A3 was first identified as a candidate tumor suppressor gene (down-regulated in adenoma [DRA]),⁶⁵ which has Cl⁻ transporter activity and is highly expressed at the luminal membrane of the intestinal epithelium. Mutations in the DRA gene cause congenital Cl⁻ diarrhea.^{66,67} Melvin et al⁶⁸ showed that DRA functions as an electroneutral, Na⁺-independent Cl⁻/HCO₃⁻ exchanger in the colon. Similarly, the guinea pig DRA protein was found to be electroneutral.⁶⁴ In contrast, it has been demonstrated by Ko et al⁴² and by Shcheynikov et al⁶⁹ that SLC26A3 functions as electrogenic 2Cl⁻/HCO₃⁻ exchanger in transfected HEK293 cells. Putative anion transporter 1 (PAT-1) was identified as a mouse kidney protein with Cl⁻/formate exchange activity.⁷⁰ It is a major apical Cl⁻/HCO₃⁻ exchanger in the small intestine and mediates the majority of prostaglandin E-stimulated HCO₃⁻ secretion in the duodenum.⁷¹ On the basis of its localization in the apical membrane of the pancreatic duct and its function as a 1Cl⁻/2HCO₃⁻ exchanger,^{42,69} PAT-1 has been proposed to be a major contributor to apical HCO₃⁻ secretion in the pancreatic duct.^{50,62,63} The electrogenic nature of the transporter could be species-dependent as Clark et al⁷² found that although mouse SLC26A6 mediates bidirectional electrogenic oxalate/Cl⁻ exchange, human SLC26A6-mediated oxalate transport appeared to be electroneutral. In microperfused guinea pig ducts, measurements of membrane potential and Cl⁻/HCO₃⁻ exchange activity suggested a probable stoichiometry of 1:2.⁷³ It is important to note that SLC26A3 and SLC26A6 expression and function have been shown to be regulated by CFTR.⁶² Although SLC26A11 expression has also been found in pancreatic ducts, the guinea pig isoform exhibited only pH-dependent Cl⁻, oxalate, and sulfate transport, but it had no detectable Cl⁻/HCO₃⁻ exchange activity in *Xenopus* oocytes.⁶⁴ Despite marked species differences among mammalian SLC26 polypeptides present in

the pancreatic duct, the anion selectivity and substrate affinity of guinea pig SLC26 anion exchangers are generally similar to those of their human orthologs, but they differ in some of their pharmacological properties.⁶⁴

Na⁺/HCO₃⁻ Cotransporter

HCO₃⁻ accumulation across the basolateral membrane of PDECs is mainly mediated by NBC, a member of the SLC4 family. NBC activity was first identified in the salamander *Ambystoma tigrinum* kidney⁷⁴ and since has been demonstrated functionally in numerous other cell types including pancreas,⁷⁵⁻⁷⁷ colon,⁷⁸ liver,^{79,80} and heart.⁸¹ The crucial role of NBC in HCO₃⁻ secretion is based on studies of isolated rat and guinea pig pancreatic ducts.⁷⁵⁻⁷⁷ Ishiguro et al⁷⁷ showed that NBC contributes to approximately 75% of the HCO₃⁻ uptake by guinea pig PDECs during stimulation with secretin. Furthermore, it has been documented that under resting conditions NBC mediates cellular HCO₃⁻ efflux when the basolateral membrane potential is about -70 mV⁸²; however, under secretin-stimulated conditions, the cotransporter mediates HCO₃⁻ influx.⁸³ The basolateral NBC isoform cloned from human pancreas and named pNBC1 by Abuladze et al¹⁹ transports 1 Na⁺ and 2 HCO₃⁻ in pancreatic ducts, but its stoichiometry is cell-type dependent⁸⁴ and can be altered by PKA phosphorylation.⁸⁵ All members of the superfamily of Na⁺-driven HCO₃⁻ transporters were discovered and classified by Boron et al,⁸⁶ who renamed it to NBCe1-B and identified 3 different splice variants (NBCe1-A, NBCe1-B, and NBCe1-C).⁸⁶

NBCe1-B, which is sometimes called pNBC1, is predominantly expressed in the pancreas.¹⁹ NBCe1-B is an electrogenic transporter that uses the Na⁺ gradient more efficiently than NHE1 to accumulate cytosolic HCO₃⁻, and indeed, NBCe1-B transports the bulk of basolateral HCO₃⁻ entry during ductal fluid and HCO₃⁻ secretion.^{5,6,77,83} The activity of NBCe1-B is regulated by multiple inputs, including IRBIT^{87,88} and the WNK/SPAK pathway.⁸⁷ NBCe1-C variant is mainly expressed in the glial cells of the brain.^{89,90}

Electroneutral NBC (named NBCn1-A or NBC3) is expressed on the luminal membrane of PDECs and plays a major role in HCO₃⁻ salvage.¹⁹ In the resting state, secretory glands absorb Na⁺ and HCO₃⁻⁹¹; however, the transporters that play part in absorbing mechanisms of these ions across the luminal membrane of the pancreatic ducts have not been characterized in great detail. Nevertheless, NBCn1-A seems to be regulated by CFTR in a cAMP/PKA-dependent manner.⁹¹ Multiprotein complexes are formed between NBCn1-A and CFTR by PDZ domain-mediated interactions, which makes it possible for CFTR to inhibit NBCn1-A activity during stimulated secretion.⁹¹ Actually inhibiting HCO₃⁻ salvage transporters during secretion is quite logical, because otherwise they would counteract the effect of secretory transporters.

Na⁺/H⁺ Exchangers

Human NHEs are members of the SLC9 gene family, which are a subgroup of the monovalent cation proton antiporter superfamily.^{92,93} NHEs are involved in numerous physiological processes, such as regulation of pH homeostasis of the cytosol and intracellular organelles. They ensure the major Na⁺-absorbing mechanism in the kidney and gastrointestinal tract.⁹⁴

NHE1 is ubiquitously expressed and is localized to the basolateral membrane of epithelial cells including PDECs.⁹⁵ NHE1 is activated by acidic pH_i levels and plays an indirect role in the mechanism of pancreatic ductal HCO₃⁻ secretion by the backward transport of H⁺ across the basolateral membrane. In most species,

the inhibition of NHE1 by amiloride has minimal effect on secretin-stimulated pancreatic ductal fluid and HCO_3^- secretion.^{96,97}

NHE2 and NHE3 are expressed in the luminal membrane of interlobular and main mouse pancreatic ducts, which are responsible for a luminal H^+ efflux (HCO_3^- salvage) mechanism.⁹⁸ In the resting state, the pH of pancreatic juice is acidic and contains high level of CO_2 , which indicate an active H^+ secretory process.⁶ To clarify the role of NHEs in this mechanism, Lee et al⁹⁸ carried out experiments by using NHE2 and NHE3 knockout mice. Approximately 45% of the luminal H^+ efflux was mediated by NHE3. Despite the expression of NHE2, its functional role could not be established. Interestingly, they identified a novel, HOE694 (amiloride analog)-sensitive, Na^+ -dependent H^+ efflux mechanism, which was responsible for the remaining (approximately 55%) luminal H^+ efflux. Importantly, CFTR is in close interaction with NHE3 and also regulates its activity.^{40,91} It is likely that the activity of NHE3 is inhibited during HCO_3^- secretion. The role of other potential NHE isoforms in ductal secretion/absorption needs further investigation.

Aquaporins

It was believed for a long time that water flow from the basolateral to the luminal side is solely driven by osmotic gradient via a paracellular pathway. However, nowadays, it is evident that water transport is also an actively mediated transcellular process. In most organisms, AQP water channels account for transcellular water permeability.^{99,100} Aquaporins are permeable not only to water, but also to small solutes such as cations and glycerol.^{99,100} There are at least 13 AQP genes (AQP0-AQP12) in mammalian cells¹⁰¹; Delporte¹⁰² gives a nice overview of pancreatic AQP expression in different mammalian species. Briefly, mouse PDECs express abundant AQP1 and AQP5 at the apical membrane and AQP1 alone at the basolateral membrane.¹⁰³ Marked expression of AQP1 and small amount of AQP5 were detected in isolated rat ductal cells by Ko et al.¹⁰⁴ They also demonstrated that AQP1 was present in both luminal and basolateral membranes of interlobular PDECs. Almost all of the secretin-evoked pancreatic fluid secretion is thought to be mediated by AQP1.¹⁰⁴ Similarly to that found in rats, human pancreatic ducts also express AQP1 in the luminal and basolateral membranes; however, AQP5 was detected only in the luminal membrane.^{105,106} Interestingly, both AQP1 and AQP5 were colocalized with CFTR at the apical membrane of intercalated duct cells.¹⁰⁵ Thus, it is no wonder that guinea pig *CFTR* gene silencing by RNA interference reduces both CFTR and AQP1 expression in PDECs, which results in inhibition of pancreatic fluid secretion.¹⁰⁷ Taken together, these observations suggest that AQP1 and AQP5 are the most important water channels in pancreatic ducts. The restoration of AQP expression by gene transfer may be beneficial as this has already been demonstrated in case of radiation-induced salivary hypofunction.¹⁰⁸

Other Enzymes, Transporters, Pumps, and Channels

Carbonic Anhydrases

Carbonic anhydrases are a diverse group of intracellular and extracellular enzymes involved in pancreatic HCO_3^- secretion. In fact, they are in close interaction and form complexes with other transporters (eg, SLC26A6, pNBC) involved in secretion.^{109,110} The nonspecific carbonic anhydrase inhibitor acetazolamide has been shown to significantly inhibit secretion.²² This may be due to a partial inhibition of basolateral HCO_3^- uptake as seen in human pancreatic duct cells.⁴⁹ Reverse transcriptase-polymerase chain reaction and immunohistochemistry confirmed the expression of carbonic anhydrase II, IV, IX, and XII in the human pancreas and/or in pancreatic ducts.^{111–113} Interestingly, the

targeting of carbonic anhydrase IV to the apical plasma membrane of duct cells seems to be CFTR dependent.^{114,115}

Na^+/K^+ -ATPase Pump and K^+ Channels

The main driving forces for pancreatic electrolyte and fluid secretion are the basolaterally expressed Na^+/K^+ -ATPase pump^{6,116,117} and K^+ channels, which produce the negative membrane potential that is essential for ductal anion secretion.^{4,118} Numerous types of K^+ channels are expressed in PDECs (including KCNN4, KCNMA1, KCNQ1, KCNH2, KCNH5, KCNT1, KCNT2, and KCNK5), which are discussed in detail by Hayashi and Novak¹¹⁸ and Venglovecz et al.¹¹⁹ Not all of these K^+ channels may be functional in the ducts, and in some cases, their localization is also a matter of question. Microelectrode and patch-clamp methods revealed functional maxi- K^+ (BK) channels, intermediate-conductance Ca^{2+} -activated K^+ (IK) channels, and pH/ HCO_3^- -sensitive K^+ channels in PDECs.¹¹⁸ Gray et al¹²⁰ have identified a Ca^{2+} -sensitive, voltage-dependent, maxi- K^+ channel on the basolateral membrane of rat pancreatic duct cells. In contrast, Venglovecz et al¹²¹ demonstrated maxi- K^+ channel expression on the luminal membrane of guinea pig PDECs. Interestingly, it has recently been shown that gastric and nongastric H^+/K^+ pumps (expressed on the luminal and basolateral membranes) may also play part in the secretion by ducts.¹²² The effects of $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (NKCC) and H^+ ATPase may be important only in rodents (rat and mice) and pigs, respectively, so they are not discussed in the current review.¹¹

Ca^{2+} -Activated Cl^- Channels

Besides CFTR, other anion channels such as Ca^{2+} -activated Cl^- channels (CaCCs) are localized on the luminal membrane of duct cells.^{123,124} Ca^{2+} -activated Cl^- channels may play role in numerous physiological processes including smooth muscle contraction and fertilization and HCO_3^- secretion in epithelial cells.¹²⁵ The molecular identity of CaCCs in PDECs needs to be investigated. A likely candidate of ductal CaCC is called ANO1 (also called transmembrane member 16A, TMEM16A, or discovered on gastrointestinal stromal tumours 1 [DOG1]), which was shown to be expressed in the CAPAN-1 human PDECs line,¹²⁶ and in centroacinar cells and small ducts of human pancreatic tissue.¹²⁷ Recent observations reported that ANO1 anion selectivity is dynamically regulated by the Ca^{2+} /calmodulin complex.¹²⁸ ANO1 becomes highly permeable to HCO_3^- at high $[\text{Ca}^{2+}]$; via Ca^{2+} -dependent interaction between ANO1 and calmodulin.¹²⁸ Other CaCC candidates in PDECs belong to the bestrophin family members. hBest1, hBest2, hBest3, and hBest4 have been identified in the CF pancreatic duct cell line, CFPAC-1.¹²⁹ hBest1 was expressed in the cell membrane and specific cytoplasmic domains and during its biosynthesis followed the classic secretory pathway.¹²⁹ Knockdown of hBest1 expression significantly decreased Ca^{2+} -activated anion efflux from CFPAC-1 cells.

REGULATION OF PANCREATIC DUCTAL SECRETION

The exocrine pancreas secretes about 1 to 2.5 L of pancreatic juice daily. Body size, but not sex, influences the rate of HCO_3^- and fluid secretion.^{130,131} The volume of secreted pancreatic fluid decreases with age, which has been confirmed by invasive^{131,132} and noninvasive techniques.¹³³ In fact, both the secretory volume and HCO_3^- output showed relatively steep decline after 20 years of age, so these need to be taken into consideration when evaluating the exocrine function of patients. The reduction in secretion may be due to age-related morphologic and functional changes of the pancreas.

The control of pancreatic secretion is divided into cephalic, gastric, and intestinal phases, the latter of which is the most important with respect to ductal secretion.⁴ Resting secretion accounts for only a small fraction of the total secreted volume. The great majority of ductal fluid is secreted in response to stimulation (eg, that induced by a meal) and is regulated by both neural (enteropancreatic vagovagal reflex) and hormonal (most importantly by secretin) components. Obviously, it is evident that pancreatic ductal secretion is very precisely regulated not only by stimulatory (Fig. 2A), but also by inhibitory (Fig. 2B) pathways.¹³⁴ Pancreatic ductal cells express many receptors for hormones and neurotransmitters, the activation of which can lead to either stimulation or inhibition of HCO₃⁻ and fluid secretion via intracellular signaling pathways detailed below. The primary signaling systems are the cAMP/protein kinase A and Ca²⁺ pathways that mediate almost all secretory gland functions.¹³⁵ An intimate interaction and crosstalk occur at multiple

levels between these 2 pathways to control and fine tune the activity of each other.¹³⁵

Stimulatory Pathways

cAMP and cGMP Signaling

Secretin, vasoactive intestinal peptide (VIP), and β-adrenergic receptor agonists are all coupled to adenylyl cyclase activation. Secretin is 1 of the most important physiological regulators of ductal HCO₃⁻ secretion. In response to the passage of food (chyme) and to low duodenal pH (between 2 and 4.5), secretin is released from enteroendocrine cells of the duodenum into the circulation and intestinal lumen.^{136,137} Other factors involved in the release of secretin include high concentration of bile salts and fatty acids.¹³⁸

The central role of secretin in stimulation of pancreatic HCO₃⁻ secretion was suggested by Chey et al,¹³⁷ who found

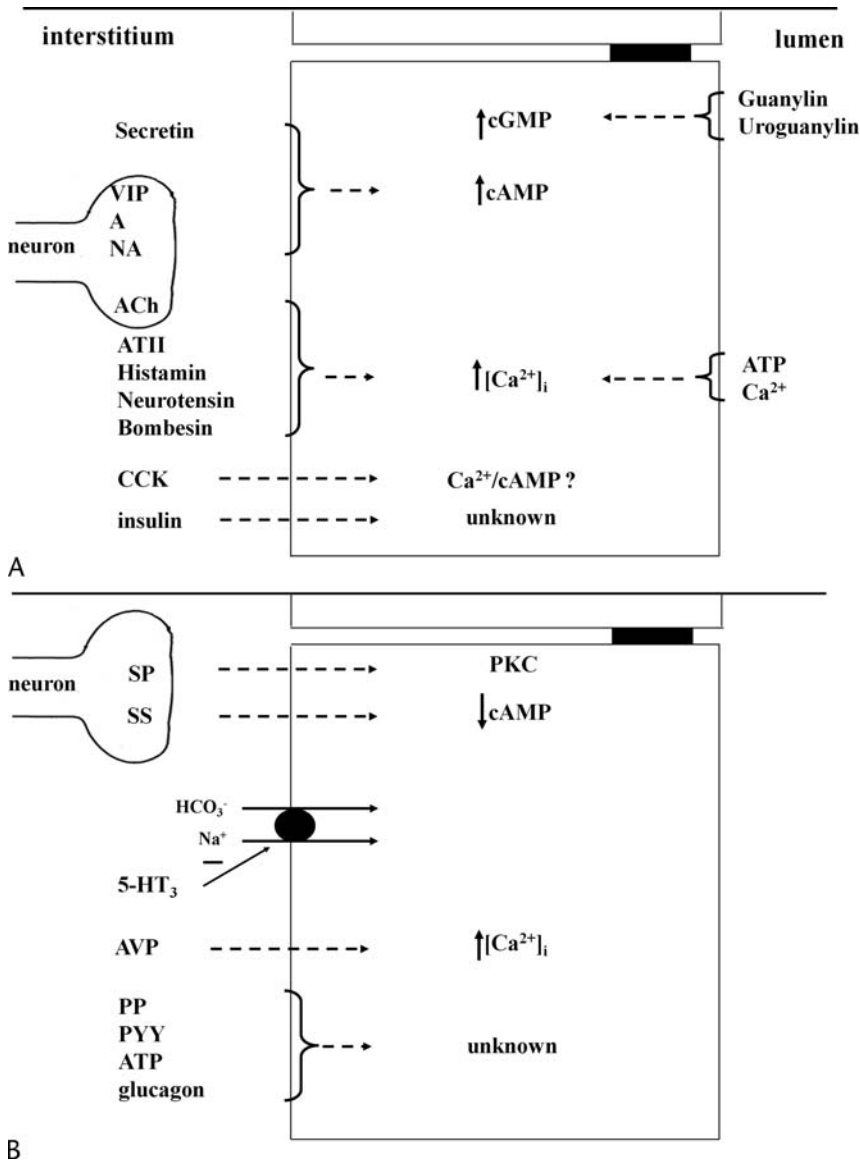


FIGURE 2. Regulation of pancreatic ductal secretion. A, Agonists which stimulate ductal secretion. B, Neurotransmitters and hormones which cause inhibition of ductal secretion. Intracellular messengers mediating their actions are shown. A, adrenaline; NA, noradrenaline; ACh, acetylcholine; ATII, Angiotensin II; CCK, cholecystokinin; SS, somatostatin; 5-HT₃, serotonin; AVP, arginine-vasopressin. Based on Argent et al.¹¹

an 80% inhibition of postprandial HCO_3^- output by administering antisecretin antibodies. It has also been proposed that other factors, such as CCK stimulation and cholinergic vagal output via an enteropancreatic vagovagal reflex, contribute to the regulation of ductal secretion.⁴ This is based on the results of Gyr et al,¹³⁹ who demonstrated that ductal secretion evoked by exogenous application of secretin is significantly lower than the extent observed during postprandial secretion. In fact, a number of publications point to the roles of CCK and vagal stimulation in secretin-induced secretion.^{140–142} Secretin stimulates pancreatic ductal fluid and HCO_3^- secretion via increasing the activity of adenylate cyclase and the level of cAMP. High intracellular cAMP level consequently activates protein kinase A,¹⁴³ which phosphorylates the regulatory (R) domain of CFTR. These events lead to the activation of CFTR and stimulation of secretion.

The secretory effects of VIP and sympathomimetics acting on β -adrenergic receptors are species dependent. Similarly to secretin, VIP increases the level of cAMP in guinea pig pancreatic ducts.¹⁴⁴ In contrast, VIP exerts weak effects on cAMP accumulation¹⁴⁵ and fluid secretion in rats.¹⁴⁶ The nonselective β -adrenergic receptor agonist isoprenaline stimulates fluid secretion in rat pancreatic ducts,¹⁴⁷ but it has no effect on cAMP concentrations in guinea pig ducts.¹⁴⁴

The intestinal peptide hormones guanylin and uroguanylin play role in the regulation of electrolyte and fluid secretion of pancreatic ducts via stimulation of guanylate cyclase C (GC-C).^{148,149} Guanylin, uroguanylin, and GC-C are expressed on the apical membrane of human and rat pancreatic ducts.^{148–150} Activation of GC-C by these peptides causes elevation of intracellular cGMP concentration.¹⁵¹ The increase in cGMP level stimulates cGMP-dependent protein kinase II,¹⁵² which mediates stimulation of CFTR¹⁵³ and finally elevates fluid and HCO_3^- secretion.^{148,154}

Ca²⁺ Signaling

Regulation of pancreatic ductal intracellular Ca²⁺ concentration ($[\text{Ca}^{2+}]_i$) is mediated by various pumps and channels.³⁶ Acetylcholine (the main neurotransmitter of the parasympathetic nervous system), ATP, angiotensin II,¹⁵⁵ and histamine¹⁵⁶ effectively stimulate ductal HCO_3^- secretion via elevation of $[\text{Ca}^{2+}]_i$.^{157,158} Furthermore, it has been shown that the Ca²⁺ ionophore ionomycin also activates ductal fluid secretion, suggesting that elevation of $[\text{Ca}^{2+}]_i$ alone is sufficient to evoke the stimulatory response.¹⁵⁷ It has been demonstrated that Ca²⁺-sensing receptor was highly expressed on the rat pancreatic duct and the luminal membrane of CAPAN-1 cells.^{159,160} Furthermore, it was also confirmed that HCO_3^- secretion is stimulated by luminal administration of Ca²⁺-sensing receptor agonist gadolinium (Gd^{3+}) via elevation of $[\text{Ca}^{2+}]_i$.¹⁵⁹

Pancreatic ducts are innervated by both peptidergic and cholinergic neurons, so it is not surprising that acetylcholine plays a role in the regulation of ductal secretion. It was shown that M2 and M3 subtypes of muscarinic receptors are present in pancreatic ducts of guinea pig, and their density is 7 times greater than that found in acinar cells.¹⁶¹ Acetylcholine directly stimulates HCO_3^- secretion in guinea pig and in rat, which is abolished by atropine and removal of extracellular Ca²⁺, and the maximal secretory response is similar to that caused by secretin.^{157,158} The $[\text{Ca}^{2+}]_i$ response evoked by acetylcholine resulted from both mobilization of Ca²⁺ stores and influx of Ca²⁺ from the extracellular space.¹⁵⁷ In addition, the cholinergic neurotransmitter potentiates the effect of secretin on secretion in isolated rat pancreatic ducts.¹⁶²

Several purines and pyrimidines found in the extracellular fluid (ie, ATP, ADP, adenosine, UTP, and UDP) can activate

intracellular Ca²⁺ signaling via purinergic receptors (P2Rs). Purinergic receptors are classified into metabotropic P2Y and ionotropic P2X receptors.¹⁶³ P2Y₂, P2Y₄, P2X₁, P2X₄, P2X₇, and probably other P2Rs such as P2Y₁ and P2Y₁₁ are expressed in pancreatic ducts.¹⁶⁴ The distribution of different receptor subtypes in pancreatic duct cells is controversial but is probably species dependent. P2Y receptors are likely localized to both apical and basolateral membranes, whereas P2X receptors are expressed only on the apical membrane.^{164,165} Ishiguro et al¹⁶⁶ demonstrated that apical and also basolateral administration of ATP evokes elevation of $[\text{Ca}^{2+}]_i$. They also showed that luminal application of ATP stimulated fluid and HCO_3^- secretion. This stimulatory effect of ATP is based on evidence that apical administration of ATP/UTP activates CFTR, $\text{Cl}^-/\text{HCO}_3^-$ exchangers, and CaCCs and also regulates K⁺ channels on CAPAN-1 cells.^{126,167} In contrast, when ATP was added from the basolateral side, the result was inhibition of either spontaneous or secretin-stimulated secretion in guinea pig pancreatic duct.¹⁶⁶ This finding was confirmed by Szűcs et al¹⁶⁸ on the human CAPAN-1 duct cells. Purinergic ligands released from nerve terminal at the basolateral membrane or from zymogen granules of acinar cells can also stimulate P2Rs.^{165,169}

The systemic renin-angiotensin system is essential for the regulation of blood pressure and electrolyte and fluid balance. In pancreatic duct cells, angiotensin II regulates anion secretion via activation of angiotensin II type 1 receptors.¹⁵⁵ It has been documented that angiotensin II dose-dependently increases short-circuit current of CFPAC-1 cell line, the effect of which is completely abolished by losartan, an angiotensin II type 1 receptor blocker and depletion of Ca²⁺.¹⁷⁰

Several other agonists (bombesin, neurotensin) can influence ductal $[\text{Ca}^{2+}]_i$ and can stimulate pancreatic secretion. For example, bombesin directly stimulates ductal HCO_3^- and fluid secretion in guinea pig via activation of gastrin-releasing, peptide-preferring bombesin receptor.¹⁵⁸ A number of publications proved that CCK increases HCO_3^- and water secretion and potentiates the effects of secretin on pancreatic ducts.^{140,158} The direct effect of CCK on guinea pig PDECs has been demonstrated by Szalmay et al,¹⁵⁸ who showed that the secreted fluid stimulated by CCK is rich in HCO_3^- and is mediated by CCK1 receptor subtypes. The effect of CCK on $[\text{Ca}^{2+}]_i$ is controversial. It has been demonstrated that CCK significantly increased cytosolic Ca²⁺ concentration up to 50-fold over baseline in rat.¹⁷¹ In another study, CCK did not cause any marked and reproducible increases in $[\text{Ca}^{2+}]_i$ on rat and guinea pig pancreatic ducts.¹⁷²

Unknown Signaling

Besides other gastrointestinal hormones, insulin also plays an important role in the regulation of ductal secretion. Initially, Hasegawa et al¹⁷³ demonstrated a potentiating effect of insulin on pancreatic juice secretion in an isolated perfused rat pancreas model. In contrast, Berry and Fink¹⁷⁴ and Howard-McNatt et al¹⁷⁵ showed that the exogenous administration of insulin inhibited secretin-stimulated pancreatic HCO_3^- secretion via a neurally mediated mechanism in dogs. The results of some other studies actually suggest that endogenous insulin promotes pancreatic secretion. Intravenous administration of glucose (resulting in elevated endogenous plasma insulin concentration) seems to increase secretin-stimulated pancreatic exocrine secretion in humans.¹⁷⁶ In accord with the latter results, stimulated pancreatic secretion was markedly blocked by treatment with rabbit anti-insulin serum, whereas it was not influenced by normal rabbit serum in rats¹⁷⁷ and dogs.¹⁷⁸ Because exogenous glucose administration (used to create systemic hyperinsulinemia via endogenous

pancreatic insulin production) did not inhibit secretin-induced pancreatic HCO_3^- secretion, Simon et al¹⁷⁹ proposed that because exogenous insulin exerts feedback regulation on the pancreas, it likely suppresses endogenous insulin secretion (which likely mediates the inhibitory response reported by Berry and Fink,¹⁷⁴ and Howard-McNatt et al.¹⁷⁵ Taken together, although exogenous insulin administration may have an inhibitory effect on ductal secretion, endogenous insulin exerts a stimulatory effect. The effect of insulin seems to be independent of changes in intracellular cAMP concentrations.¹⁴⁴

Inhibitory Pathways

The inhibitory regulation of pancreatic secretion is mediated via direct (on the ductal cells) or indirect mechanisms. The inhibition of secretion may be physiologically important in reducing secretion back to the basal level after a meal and also in maintaining the integrity of the pancreas via limiting hydrostatic pressure within the duct lumen.¹⁸⁰ This is crucial in case of ductal obstruction as the elevated pressure may seriously damage the pancreas. Unfortunately, the authors' knowledge of inhibitory mediators is scarce, especially concerning their molecular mechanisms of inhibition, but numerous substances have been shown to negatively regulate secretion, which are discussed below. For a more detailed overview of inhibitory substances, refer to the authors' earlier publication.¹⁸⁰

Substance P

The neuropeptide substance P (SP) is a potent inhibitor of pancreatic ductal HCO_3^- and fluid secretion. Substance P strongly inhibits *in vivo* pancreatic fluid secretion in multiple species such as the dog,^{181,182} rat, and mouse.¹⁸³ Moreover, SP inhibits both basal- and secretin-stimulated fluid secretion of isolated rat and guinea pig pancreatic ducts *in vitro*,^{146,184} suggesting a direct action of SP on pancreatic duct cells. The inhibitory effect of SP is dose dependent in rat and was partially reversed by spantide, a neurokinin (NK) receptor antagonist.¹⁴⁶ Accordingly SP exerts its inhibitory effect via the activation of G protein-coupled NK receptors. Kemény et al¹⁸⁵ demonstrated that all 3 NK receptors are expressed in the luminal membrane, whereas NK2 and NK3 receptors were also detected on the lateral membranes of guinea pig pancreatic ductal cells. Furthermore, both of the laterally expressed NK receptors mediate the inhibitory effect of SP on isolated guinea pig pancreatic duct.¹⁸⁵ Substance P binding to NK receptors activates PKC isoforms, which are expressed in PDECs and mediate the inhibition of HCO_3^- secretion by modulating an SLC26 $\text{Cl}^-/\text{HCO}_3^-$ exchanger.^{184,186} To confirm that the effect of SP is indeed mediated by PKC, the highly selective, cell-permeable PKC inhibitor bisindolylmaleimide was used.¹⁸⁶

Serotonin

5-Hydroxytryptamine (5-HT)-reactive cells with morphological characteristics of enterochromaffin cells are present throughout the duct system, that is, the main, intralobular, and interlobular ducts of guinea pigs.¹⁸⁷ In isolated interlobular ducts, basolateral administration of 5-HT strongly but reversibly inhibited secretin- and ACh-stimulated fluid secretion as well as spontaneous (HCO_3^- -dependent) secretion.¹⁸⁷ The inhibition is mediated by the 5-HT₃ receptor, a ligand-gated, nonselective cation channel. Luminal administration of 5-HT failed to affect basal and secretin-stimulated fluid secretion, suggesting that only basolateral, but not luminal, 5-HT receptors mediate the inhibition of fluid secretion.¹⁸⁷ The inhibition is probably due to the reduced uptake of HCO_3^- via $\text{Na}^+-\text{HCO}_3^-$ cotransport across

the basolateral membrane. The enterochromaffin cells in the pancreatic duct may function as intraductal pressure sensors and regulate ductal fluid secretion. When the intraluminal pressure of pancreatic ducts increases, 5-HT is released into the interstitium from the ductal enterochromaffin cells, and the released 5-HT binds to 5-HT₃ receptors on the basolateral membrane of duct cells and inhibits fluid secretion.¹⁸⁷ This may be a key mechanism in maintaining the integrity of the pancreatic tissue.

Arginine Vasopressin

Arginine vasopressin plays a key role in the fluid homeostasis of mammals. In the pancreas, 2 early publications suggested that arginine vasopressin inhibits pancreatic secretion in an indirect manner.^{188,189} Beijer et al¹⁸⁸ demonstrated that the vasoconstriction caused by arginine vasopressin decreases blood flow and reduces the oxygen consumption of the pancreas in anesthetized dogs. Few years later, Kitagawa et al¹⁸⁹ showed that exogenous administration of vasopressin caused dose-dependent inhibition of pancreatic juice flow and HCO_3^- output by elevation of plasma osmolality in conscious dog. Furthermore, arginine vasopressin also inhibits secretin-stimulated fluid secretion in isolated guinea pig pancreatic ducts via elevation of $[\text{Ca}^{2+}]_i$ from intracellular Ca^{2+} stores.¹⁹⁰

Somatostatin

Somatostatin is secreted from several locations including the gastrointestinal tract (eg, the stomach, the intestine, and the delta cells of pancreas) and the central nervous system. Somatostatin was first identified in the brain by Brazeau et al¹⁹¹ in 1973, and its function was related to inhibition of growth hormone secretion; thus, this peptide is also known as a growth hormone-inhibiting hormone. Since then, it has been demonstrated that somatostatin has a wide range of inhibitory functions. The exogenous administration of somatostatin inhibited pancreatic HCO_3^- secretion induced by meal and also reduced the secretin-stimulated pancreatic HCO_3^- secretion.^{173,192–196} Konturek et al¹⁹⁵ demonstrated that the somatostatin analog cyclosomatostatin caused dose-dependent inhibition of pancreatic HCO_3^- secretion via partially direct inhibitory effect on exocrine pancreas and the reduction of secretin release in dogs. The indirect inhibitory mechanism of somatostatin was confirmed by the observations of Kuvshinov et al,¹⁹⁷ who demonstrated the role of intrapancreatic cholinergic mechanism in the inhibitory effect of somatostatin on secretin-stimulated HCO_3^- secretion. Furthermore, somatostatin significantly reduced the effects of secretin on cyclic AMP level of pancreatic duct cells via inhibition of adenylyl cyclase activity.¹⁴⁴

Pancreatic Polypeptide and Peptide YY

Pancreatic polypeptide (PP) and peptide YY (PYY) are structurally related peptide hormones. In fact, PP is derived from duplication of the PYY gene.¹⁹⁸ Both PP and PYY are released in response to intake of food.¹⁹⁹ Pancreatic polypeptide is secreted by PP cells of the Langerhans islets.²⁰⁰ It has been shown that the physiologic function of PP is to inhibit pancreatic HCO_3^- secretion in response to meal and secretin.^{201–203} Konturek et al²⁰¹ demonstrated marked differences in the effect of PP on the exocrine pancreas of man and dog. Pancreatic polypeptide administration caused dose-dependent inhibition of secretin-stimulated pancreatic fluid and HCO_3^- secretion in dog, but not in human. Similarly to this observation, Lonovics et al²⁰² have also shown that PP reduced the endogenously stimulated pancreatic secretion in a dose-dependent manner, whereas the release of CCK and secretin was not affected. Based on these results, they suggested that

the inhibitory effect of PP is probably direct and that it is not mediated via inhibition of CCK or secretin release.²⁰²

Peptide YY is secreted by L cells localized in the mucosa of gastrointestinal tract, especially in ileum and colon.²⁰⁴ It plays fundamental roles in numerous physiological processes, including inhibition of gastric acid and meal-stimulated pancreatic fluid and HCO_3^- secretion; furthermore, it increases water and electrolyte absorption in the colon.^{205,206} Exogenous application of PYY also reduced secretin- and CCK-stimulated secretion in dog.²⁰⁷ The inhibitory action of PYY on pancreatic secretion is likely to be indirect; thus, it is fully mediated by the vagal efferent nerve.²⁰⁸

Glucagon

Glucagon is secreted by alpha cells of the Langerhans islets and is known to have an essential role in the regulation of glucose metabolism. Besides this important function, the exogenous administration of glucagon also inhibits stimulated pancreatic HCO_3^- secretion in rats,¹⁷³ dogs,^{209–212} cats,²¹³ and humans.^{214,215} Generally speaking, the inhibitory effect of glucagon on digestive enzyme output is greater than that on pancreatic secretory volume and bicarbonate output. Glucagon had no effect on the levels of endogenously released secretin.²¹¹ This observation suggests that the inhibitory effect of glucagon on pancreatic secretion is not mediated via inhibition of secretin release. In addition, glucagon did not significantly alter resting or secretin-stimulated cyclic AMP levels in isolated guinea pig pancreatic duct segments.¹⁴⁴

Regulatory Proteins Involved in Epithelial Fluid and HCO_3^- Secretion

PDZ-Based Adaptors

Numerous PDZ domain-containing transporters play a fundamental role in the HCO_3^- transport mechanism of pancreatic ducts via formation of protein complexes. PDZ stands for the first letters of 3 proteins that were initially shown to possess such domains: postsynaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (Dlg1), and zonula occludens 1 protein (ZO-1). PDZ domain is a common structural unit of 80 to 90 amino acids that mediates protein-protein interactions by binding to short peptide sequences, most often in the C termini of target proteins.²¹⁶ PDZ domains are responsible for targeting and trafficking of several membrane proteins such as receptors, transporters, channels, and adhesion proteins, through their PDZ-binding motifs.²¹⁷ Furthermore, they bind to the PDZ domains of other proteins and develop multiprotein scaffolding networks.²¹⁷

One of the PDZ proteins that is important in epithelial transport is the NHERF family. NHERF-1 (also known as ezrin-binding protein of 50 kd [EBP50]) is a scaffolding protein, which tethers several membrane protein to apical actin cytoskeleton in polarized epithelia via ezrin.²⁸ The adapter protein has been shown to bind to the PDZ-binding motifs of CFTR Cl^- channel, NHE3, β_2 -adrenoreceptor,^{218,219} and Slc26 family anion exchangers (Slc26A3)²²⁰ and PAT-1 (Slc26A6).⁶³ In addition, NHERF-1 facilitates the formation of multiprotein complexes, which is fundamental for the adequate function of transporters, channels, and receptors.⁴³ Therefore, it is not surprising that NHERF-1 is involved in numerous physiological processes such as the regulation of phosphate transport in the kidney,²²¹ hepatic Mrp2 expression and function,²²² protein kinase D activity,²²³ or trafficking of β_2 -adrenergic receptors.²²⁴ To confirm the role of NHERF-1 in the pancreas, we demonstrated that the genetic deletion of NHERF-1 greatly reduced the translocation of CFTR to the luminal pancreatic

ductal cell membrane and also decreased both in vitro and in vivo pancreatic HCO_3^- and fluid secretion.²²⁵ Other studies have identified the fundamental role of NHERF1 and NHERF2 in the regulation of the luminal HCO_3^- salvage transporters NHE3 and NBCn1-A via formation of multiprotein complexes with CFTR.^{40,91,226} This interaction may also be important in inhibition of these salvage transporters during secretion.

Moreover, pancreatic duct cells express several other scaffold proteins with PDZ domains, such as Shank2, S-SCAM, SAP97, and PSD-95.^{6,216,217} Shank2 is localized to the apical pole of pancreatic duct cells and is involved in the regulation of the expression and activities of CFTR and NHE3.^{6,216,227}

With-No-Lysine and Sterile 20-Like Kinases

Recent publications suggest that WNK and SPAK have essential roles in the regulation of salt homeostasis and blood pressure via modulation of the activity of diverse ion transporters.^{228–230} In fact, the main function of WNKs is the regulation of Na^+ , K^+ , Cl^- , HCO_3^- , and Ca^{2+} transporters in epithelia^{231–233} either by modulating their surface expression via promoting their endocytosis or by regulating their activity.^{12,234} It is likely that WNKs do not act directly on the ion transporters, but they activate downstream kinases SPAK and OSR1.⁶ The activated SPAK/OSR1 phosphorylates the ion transporters and evokes their endocytosis.²³⁰

In pancreatic ducts, WNKs act through SPAK to control the activities of NBCe1-B and CFTR, and knockdown of WNKs and SPAK increases pancreatic ductal secretion.²³⁵ The WNK/SPAK pathway appears to have dual function in pancreatic ducts in the resting and stimulated states.⁶ Under resting conditions, WNK/SPAK pathway reduces surface expression and activity of transporters (such as CFTR, SLC26 anion exchangers, and NBCe1-B), which will overall reduce pancreatic ductal fluid and HCO_3^- secretion.^{34,87,235,236} In the stimulated state, when $[\text{Cl}^-]_i$ is low (in the distal ducts), the WNK/SPAK pathway has an opposite effect. In this case, the activation of the WNK1/SPAK resulted in increased HCO_3^- permeability of CFTR (making it primarily an HCO_3^- channel) and inhibited apical $\text{Cl}^-/\text{HCO}_3^-$ exchange activity (that may reabsorb HCO_3^- from the lumen).^{6,34}

IP3 Receptor–Binding Protein Released With IP3

IRBIT was identified as a protein that interacts with the IP₃-binding domain of IP₃ receptors (IP₃R).²³⁷ It suppresses the activation of IP₃R and inhibits IP₃-induced Ca^{2+} release by competing with IP₃ binding on the NH₂-terminal domain of the IP₃ receptor.^{238,239} Besides its other diverse functions, accumulating evidence from the groups of Muallem and Mikoshiba suggests that IRBIT has an essential role in the regulation of epithelial HCO_3^- secretion.^{37,87,88,240,241} IRBIT aggregates at the apical pole of the pancreatic duct,⁸⁷ where expression of IP₃R is also high. IRBIT antagonizes the effect of the WNK/SPAK pathway and stimulates ductal secretion in 2 ways: it increases the cell surface expression and also the activities of Cl^- and HCO_3^- transporters.^{12,235} It has been reported that IRBIT interacts with and regulates the activities of CFTR, SLC26A6 and possibly NHE3 on the apical pole, and NBCe1-B on the basal part of the ductal cells. The exact regulatory mechanism mediated by IRBIT is only partly understood, but it seems that IRBIT activates basolateral and apical transporters by different mechanisms. Shirakabe et al⁸⁸ demonstrated that IRBIT induces conformational changes in pNBCe1-B, which results in dissociation of its autoinhibitory domain. In contrast, IRBIT activates CFTR by direct interaction and reduces the close-duration time of CFTR and thus increases CFTR open probability.^{6,87} Importantly, IRBIT also acts as a conductor to mediate synergism between Ca^{2+} and

cAMP signaling pathways in regulating the activation of CFTR and SLC26A6.³⁷

PATHOPHYSIOLOGICAL ROLE OF PANCREATIC DUCTAL SECRETION

Cystic Fibrosis

The secretory function of the pancreatic ductal system is rather markedly affected by CF, which is the most common fatal autosomal recessive disease in white population. Cystic fibrosis is caused by the absence or dysfunction of CFTR, a critical regulator of HCO₃⁻ secretion. Almost 2000 CF-causing mutations have been identified in the *cftr* gene.²⁴² Mutations are divided into 6 different classes according to mechanisms of CFTR dysfunction: defective protein production (I), defective protein processing (II), defective channel regulation (III), defective channel conduction (IV), reduced protein synthesis (V), and reduced protein stability (VI). The most common *cftr* mutation, the deletion of phenylalanine at position 508 (delF508-CFTR), was identified in 1989.²⁴ This mutation primarily results in CFTR misfolding and degradation (class II), but it also shows other defects of classes III and VI.^{27,28,242–244} Although the life expectancy of individuals with CF has increased dramatically in the past decades, the average age of death (caused by respiratory failure) is still about 40 years.²⁴⁵ The disorder affects all epithelia expressing CFTR and presents with exocrine pancreatic insufficiency, an increase in sweat NaCl concentration, male infertility, and airway disorder.²⁸ The major defect in CF is the inability to maintain luminal hydration of the lung, pancreas, and epididymis, which leads to thick and viscous fluid formation plugging the affected organs that will cause massive damage.^{246,247} The deficient ductal electrolyte and fluid secretion causes the pancreatic juice of CF patients to become acidic.⁴ This will contribute to precipitation of mucins and premature digestive enzyme activation inside the ductal lumen, resulting in the characteristic pancreatic cyst formation and scarring (fibrosis).²⁴⁸ The destruction of acinar cells eventually leads to pancreatic insufficiency, the degree of which can range from mild to severe depending on the *cftr* mutation.^{249,250}

Because at least part of HCO₃⁻ secretion is coupled to Cl⁻ transport, it is no wonder that *cftr* mutations resulting in reduced Cl⁻ conductance can markedly impact the secretory process. However, a number of disease-causing *cftr* mutations were found that support normal or even elevated Cl⁻ channel activity, but had no (in pancreatic insufficient patients) or

reduced (in pancreatic sufficient patients) HCO₃⁻ transport.³⁹ This may be due to defective activation of SLC26 anion exchangers catalyzing HCO₃⁻ secretion by mutant CFTR.⁴² These results also suggest that in some cases correction of Cl⁻ transport may not necessarily be enough to restore HCO₃⁻ secretion.

Recently, small-molecule drug-like CFTR correctors and potentiators have been utilized in clinical trials involving CF patients to compensate for trafficking and gating defects, respectively.^{251,252} Cystic fibrosis transmembrane conductance regulator correctors (eg, 4-phenyl-butyrate or VX-809) are targeted at protein misprocessing, whereas potentiators (eg, ivacaftor) are used to restore Cl⁻ channel activity. Some compounds have both corrector and potentiator effects, which could be especially useful in treating the most common form (F508del) of *cftr* mutation. Gene transfer-based therapies (to introduce wild-type CFTR into cells expressing defective CFTR) have also offered hopeful perspectives in resolving the problem of CF. Because the majority of the morbidity and mortality in CF is a result of lung disease, most efforts in this field have focused on gene transfer to the airway. We have shown that wild-type *cftr* transfer using a recombinant Sendai vector significantly increased the apical Cl⁻/HCO₃⁻ exchange activity of CFPAC-1 duct cells derived from the adenocarcinoma of a CF patient.⁵⁰ Therefore, restoration of pancreatic ductal secretion by wild-type CFTR should be beneficial in CF patients. Because the receptor for the Sendai virus is localized to the apical membrane of PDECs, a retrograde injection of this vector into the pancreatic duct would be necessary in vivo. This is not necessarily ideal, so other vectors may be more appropriate in patients. For example, Griffin et al²⁵³ have successfully transduced PDECs of newborn pigs through the umbilical artery with an adenoassociated virus serotype 9 vector.

To study the pathogenesis and therapy of CF, most commonly murine models are used. Although we have learned a lot from using CFTR knockout mice, the utilization of these animals has numerous limitations such as anatomic, immunologic, and disease phenotypic differences between human and mice.^{254–256} Importantly, CF mice exhibit milder pancreatic pathology compared with patients.²⁵⁵ This may be due to residual CFTR activity and/or the presence of alternate Cl⁻ secretory pathways such as Ca²⁺-activated Cl⁻ channels. In fact, patch clamp experiments have shown that Ca²⁺-activated Cl⁻ conductance is much greater than that caused by CFTR in mouse duct cells.¹²⁴ Human pancreatic duct cells also express Ca²⁺-activated Cl⁻ channels at high levels,²⁵⁷ which makes them a potential therapeutic target in CF patients. To complicate things further, pancreatic ducts isolated from CFTR-null mice still secreted electrolytes and fluid, which

TABLE 1. Disease Phenotypes of the Pig and Ferret Cystic Fibrosis Models Compared With the Human Disease in Newborns

	Pig	Ferret	Human
Lung histology	Normal	Infected by streptococci and staphylococci	Normal
Pancreatic destruction	Very severe	Mild	Mild
Meconium ileus	100%	75%	15%
Liver	Focal biliary cirrhosis	Histologically normal, but elevated LFT	Histologically normal, but elevated LFT
Gallbladder	Mucus- and bile-filled microgallbladder	Normal	30% Microgallbladder
Vas deferens	Intact	Absent or degenerate	Intact

Newborn pigs and ferrets lacking CFTR exhibit defective chloride transport which leads to numerous alterations in different organs affected by cystic fibrosis. Notably, tissue damage progresses throughout the life of the diseased animals/humans.

LFT indicates liver function tests.

could not be attributed to Ca^{2+} -activated Cl^- channels.²⁵⁸ Chelation of intracellular Ca^{2+} did not influence stimulated fluid secretion in CF versus wild-type ducts. Further studies are needed to identify the mechanism responsible for the CFTR-independent fluid transport.

The recent development of CF pigs²⁵⁹ and CF ferrets²⁶⁰ provides better disease models as they show more similarities to the human CF phenotype. Newborn pigs and ferrets lacking CFTR exhibited defective chloride transport and develop meconium ileus, pancreatic pathology, and liver involvement (focal biliary cirrhosis or elevated liver function tests), mimicking abnormalities seen in newborn humans with CF (Table 1). Meconium ileus is potentially lethal unless treated by surgery. Seventy-five percent of the CFTR-deficient ferrets failed to pass meconium and died within 48 hours of life because of intestinal perforation and sepsis.²⁶⁰ Similarly, CF pigs required ileostomy to relieve meconium ileus to live beyond the first few days after birth.²⁵⁹ Fetal and newborn CF pigs had very severe pancreatic destruction (whereas most CF patients at this stage have only mild lesions) and exhibited significantly lower volume and pH of pancreatic fluid, whereas protein concentration was more than 5-fold higher versus the control.²⁶¹ They also had significantly increased expression of proinflammatory, complement cascade, and profibrotic genes versus non-CF pigs.²⁶² Furthermore, their acinar cells exhibited a higher apoptosis rate. Newborn ferrets have only mild pancreatic pathology of duct dilations with inspissated secretions (Table 1). Juvenile and adult CF ferrets develop more severe pancreatic pathology, which is similar to CF patients, but 15% of the animals retain predominantly normal pancreatic histology at the time of death.²⁶³ This suggests that additional factors may be necessary for the manifestation of the disease. Although CF pigs and ferrets seem to be more useful and promising models compared with mice, the problems with these animals include limited access for most researchers and the severe intestinal phenotypes.

It is known that pancreatic sufficient patients with CF have the greater risk of developing pancreatitis.^{264,265} Not surprisingly, the type of CFTR mutation determines the risk of pancreatitis²⁶⁵; CF patients with mild CFTR genotypes have a greater risk of developing pancreatitis compared with patients with moderate-severe genotypes. Pancreatitis occurred in 20% of the patients, with a quarter presenting with pancreatitis prior to the diagnosis of CF, typically in late childhood and adulthood.²⁶⁵ The majority (60%) of affected CF patients experienced recurrent attacks of acute pancreatitis; 18% had a single episode of acute pancreatitis, and 22% showed signs of chronic pancreatitis. Furthermore and importantly, patients with pancreatitis received a diagnosis of CF at a significantly older age and had lower sweat chloride levels than did patients without pancreatitis.²⁶⁵

Acute Pancreatitis

Acute pancreatitis is a sudden inflammation of the pancreas without any reliable treatment. There are several diverse causative factors for pancreatitis, including biliary disease, excessive ethanol intake, and metabolic causes such as hypertriglyceridemia.^{266–268} The severity of acute pancreatitis can vary from mild to severe.²⁶⁹ The exact pathomechanism of acute pancreatitis is not well understood; however, animal models provide an opportunity for the investigation of pathophysiological processes and for the development of new therapeutic possibilities.²⁷⁰ Almost all types of acute pancreatitis seem to share a common mechanism of abnormal inhibition of pancreatic zymogen secretion and premature activation of these digestive enzymes.²⁷¹ In addition or alternatively, stressors inducing pancreatitis can also activate nuclear factor κB ,²⁷² a transcription factor responsible for regulating the expression of numerous proinflammatory mediators.

Although acute pancreatitis has long been considered as a disease of the acinar cells, recent evidence has proved the role of pancreatic ducts in the pathogenesis of the disease. In fact, they may be primary targets of toxic factors. Bile acids and ethanol dose dependently affect pancreatic ductal HCO_3^- and fluid secretion (Figs. 3 and 4).^{273–275} When nonconjugated bile acids are administered luminally to isolated guinea pig ductal cells in low concentration (100 μM), they activate luminal $\text{Cl}^-/\text{HCO}_3^-$ exchange activity and thus stimulate HCO_3^- secretion via inducing oscillatory elevation of intracellular Ca^{2+} concentration (Fig. 3A).²⁷⁴ This stimulatory effect of chenodeoxycholate was also confirmed in human CFPAC-1 cell line and was shown to be dependent on CFTR expression, but not CFTR Cl^- channel activity.²⁷⁶ We believe that the stimulation of pancreatic secretion may serve as a defense mechanism of ductal cells, so toxic bile acids are washed out from the ductal tree to protect acinar cells. If this process is inefficient, bile acids will reach the ductal cells in high concentration and cause sustained (eventually toxic) elevation of intracellular Ca^{2+} signaling, damage the mitochondria, deplete intracellular ATP levels, and consequently block basolateral and apical ion transport mechanisms (Fig. 3B).^{36,273,274,277} The results of the authors' recent preliminary experiments have suggested that there is also reduction of pancreatic ductal HCO_3^- secretion in patients with biliary acute pancreatitis.²⁷⁸ Intraductal pH was significantly lower in these patients versus control subjects.

One of the most common causes of acute pancreatitis is excessive ethanol consumption. Interestingly, ethanol administration itself does not induce experimental acute pancreatitis in rodents.^{279,280} Ethanol exerts only modest effects on acinar cell Ca^{2+} homeostasis, even in very high concentrations, whereas a combination of alcohol and fatty acids causes massive intracellular Ca^{2+} release and intracellular trypsinogen activation.^{279–283} There are fewer data available concerning the effects of ethanol and their metabolites on pancreatic ductal cells. Similarly to nonconjugated bile acids, ethanol has a dual effect on pancreatic HCO_3^- secretion. Yamamoto et al²⁷⁵ showed that ethanol in low concentration augments the stimulatory effect of secretin (Fig. 4A), whereas in high concentration, it inhibits the secretory rate (Fig. 4B). This dual effect may be partly mediated by CFTR. Electrophysiological studies on native guinea pig pancreatic ductal cells showed that 10 and 100 mM ethanol increases basal, but reversibly blocks, forskolin-stimulated CFTR currents via depletion of ATP_i .²⁸⁴

There is evidence that the premature activation of trypsinogen to trypsin inside the acinar cell^{285,286} or in the ductal lumen^{287,288} is a key event in the development of pancreatitis. A number of publications proved that trypsin activates enzyme secretion from acinar cells via proteinase-activated receptor 2 (PAR-2),^{289,290} but the effect of trypsin on PDECs is somewhat different depending on the species and also on the localization of PAR-2 (Fig. 5). Nguyen et al²⁹¹ suggest that trypsin activates CFTR and Ca^{2+} -activated Cl^- channel in dog PDECs and stimulates HCO_3^- secretion in the CAPAN-1 human pancreatic adenocarcinoma cell line.¹⁶⁷ In contrast, HCO_3^- efflux is inhibited by trypsin in a dose-dependent manner in bovine PDECs.²⁹² We demonstrated that PAR-2 activation by trypsin inhibits both the SLC26 anion exchanger(s) and CFTR Cl^- channel in guinea pig PDECs.²⁹³ Trypsin stimulates HCO_3^- secretion via PAR-2 activation, when the receptor is localized to the basolateral membrane.^{167,291} In contrast, the effect is inhibition when the receptor is localized to the luminal membrane.^{292,293}

The role of PAR-2 in experimental acute pancreatitis is controversial and is greatly dependent on the utilized disease model. Numerous studies have confirmed that PAR-2 has a

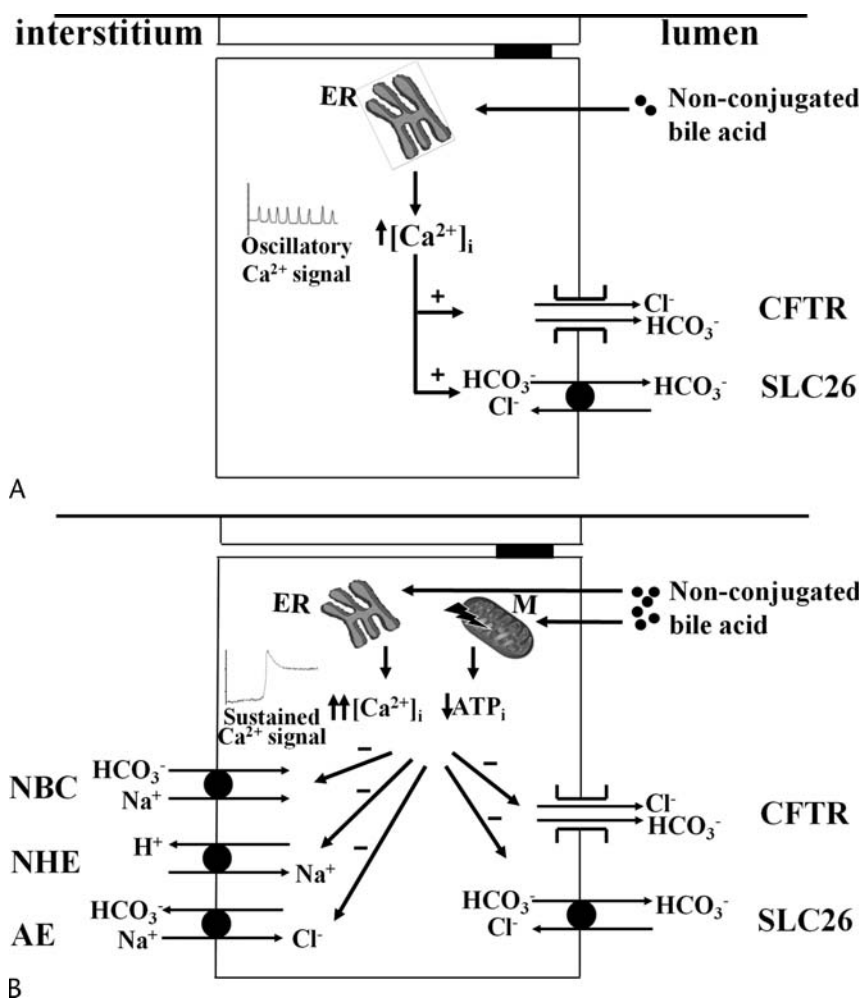


FIGURE 3. Effects of nonconjugated bile acids on pancreatic ductal HCO_3^- secretion. Bile acids may enter the pancreatic duct in case of distal biliary obstruction. A, Low doses of bile acids induce dose-dependent Ca^{2+} release from intracellular Ca^{2+} stores, and stimulate HCO_3^- secretion via activation of luminal $\text{Cl}^-/\text{HCO}_3^-$ exchanger. B, High concentration of bile acids induce a toxic, sustained $[\text{Ca}^{2+}]_i$ elevation and ATP_i depletion, which inhibit the acid-base transporters on the basolateral and luminal membrane of ductal cells. ER, endoplasmic reticulum; M, mitochondrion; +, stimulation; -, inhibition.

protective role in secretagogue-induced pancreatitis in mice^{294–296} and rats.²⁹⁷ Based on results obtained in transgenic mice, Singh et al²⁹⁰ have hypothesized that PAR-2 activation may decrease secretagogue-induced pancreatic damage by promoting zymogen secretion from acinar cells. Namkung et al²⁹⁸ demonstrated that down-regulation of MAP kinase signaling pathway may be involved in the protective effects of PAR-2 activation. However, in contrast to that found in the cerulein-induced acute pancreatitis model, deletion or pharmacological inhibition of PAR-2 markedly reduced the severity of pancreatitis evoked by intraductal infusion of taurocholate in mice,^{295,299} which implies a detrimental role of this receptor. The previously mentioned discrepancies may be due to differential local and systemic effects of PAR-2 activation.²⁹⁷ Interestingly, we have demonstrated that PAR-2 is also localized to the apical membrane of human and guinea pig PDECs. Furthermore, PAR-2 activation reduced pancreatic ductal HCO_3^- secretion by inhibition of the apical anion exchanger and CFTR.²⁹³ The reduction in ductal secretion can facilitate the development of pancreatitis by decreasing luminal pH and promoting premature activation of trypsinogen inside the pancreatic ducts.

Recently, it has been demonstrated that smoking is an important risk factor for non-gallstone-related acute pancreatitis.³⁰⁰ The risk is even higher in individuals with associated monthly ethanol consumption of 400 g or greater. The effect of cigarette smoke may be partly mediated by impairment of CFTR function.^{301–303} Clunes et al³⁰⁴ proposed that cigarette smoke exposure rapidly reduces CFTR function by internalizing CFTR protein, leading to airway dehydration. Similar mechanisms of CFTR inhibition are suspected in the pancreas; however, no detailed investigations have been performed in this research field. The detrimental effect of cigarette smoke is potentially caused by the combined effects of several thousand chemicals, and it is difficult to pinpoint individual compounds. Nevertheless, some studies have attempted to investigate the effects of major cigarette components. Nicotine was found to inhibit pancreatic HCO_3^- secretion in the dog,³⁰⁵ but others could not confirm these findings.³⁰⁶

Notably, some recent publications have identified the role of CFTR in the pathogenesis of acute pancreatitis, which provide strong evidence for the involvement of pancreatic ducts. DiMagno et al^{307,308} found that CFTR knockout mice exhibited more severe acute pancreatitis than did wild-type mice.

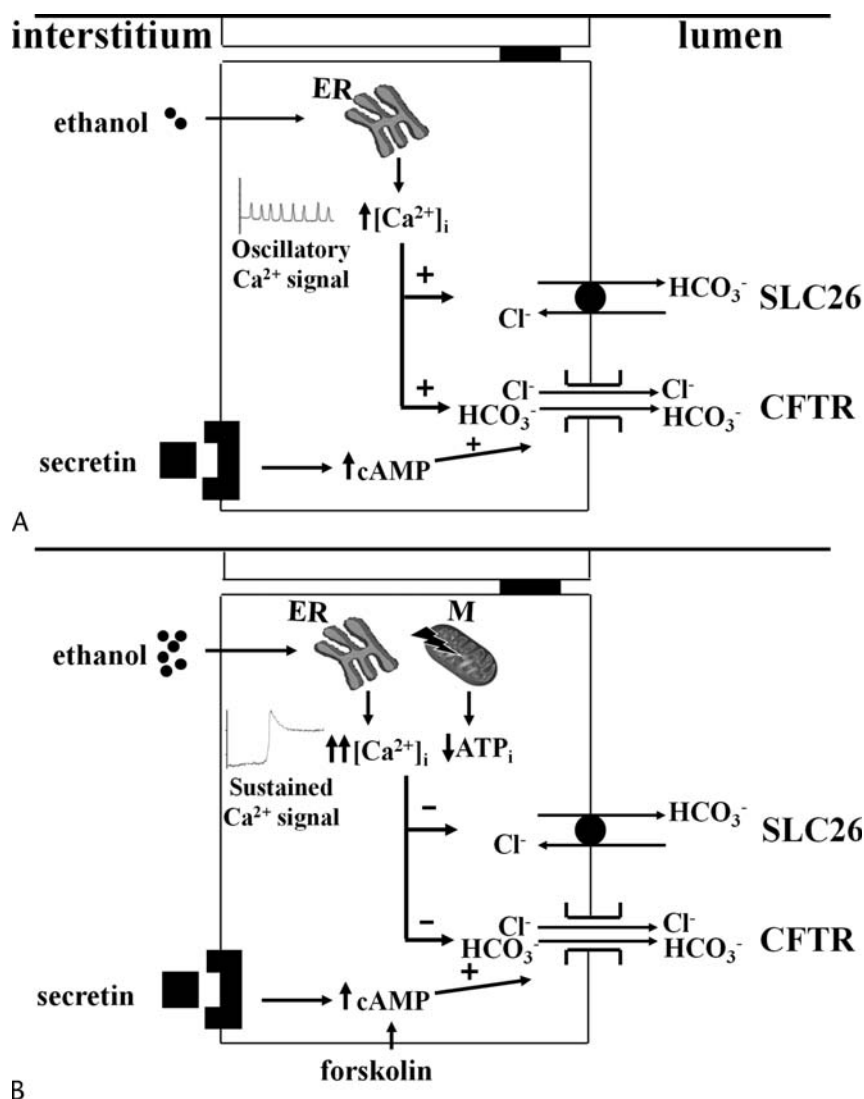


FIGURE 4. Effects of ethanol on pancreatic ductal HCO_3^- secretion. A, Ethanol and secretin together cause elevation of $[\text{Ca}^{2+}]_i$ and cAMP level. Ethanol in low concentration enhances secretin-stimulated HCO_3^- secretion. B, Ethanol in high concentration inhibits secretin- and forskolin-stimulated HCO_3^- secretion by releasing Ca^{2+}_i and depletion of ATP_i . ER, endoplasmic reticulum; M, mitochondrion; –, inhibition; +, stimulation.

However, *CFTR* knockout animals are also characterized by exuberant pancreatic inflammation, impaired acinar apoptosis, and mild pancreatic insufficiency,^{307,308} which could potentially confound the obtained results. Furthermore, only the secretagogue-induced acute pancreatitis model was tested, so model-specific effects cannot be excluded. Recently, we have demonstrated that NHERF-1 knockout mice, which have markedly lower pancreatic ductal HCO_3^- and fluid secretory rates due to a reduction of apical *CFTR* expression, also show signs of more severe acute pancreatitis in 2 disease models versus wild-type mice.²²⁵ Furthermore, Cavestro et al³⁰⁹ identified statistically significant association of *CFTR* mutations with acute recurrent pancreatitis, which may be due to insufficient pancreatic electrolyte and fluid secretion.³¹⁰

Chronic Pancreatitis

Chronic pancreatitis is a progressive inflammatory disorder that leads to irreversible destruction of the pancreas.

Pancreatic damage may result from repeated attacks of acute pancreatitis,^{311,312} but some doubt that this is in fact the case. On histology, the defining triad of chronic pancreatitis (irrespective of the cause or location) is acinar loss, mononuclear cell infiltration, and interlobular/intralobular/periductal fibrosis.³¹³ The inflammatory milieu also leads to alteration of endocrine functions.³¹⁴ Overall, these changes eventually lead to exocrine and endocrine pancreatic insufficiency, which manifest in steatorrhea, malnutrition, and type 3c diabetes mellitus.

The pancreatic ductal tree is affected both morphologically and functionally in chronic pancreatitis. These changes are essential in the diagnosis of the disease and can be nicely followed by pancreatic imaging and function tests. Ducts become dilated with concretions, and the epithelium shows signs of atrophy or hyperplasia or undergoes squamous metaplasia. It is no wonder that chronic pancreatitis greatly increases the risk of pancreatic ductal adenocarcinoma.³¹⁵ It has long been known that age at diagnosis, smoking, and drinking are major predictors of progression and

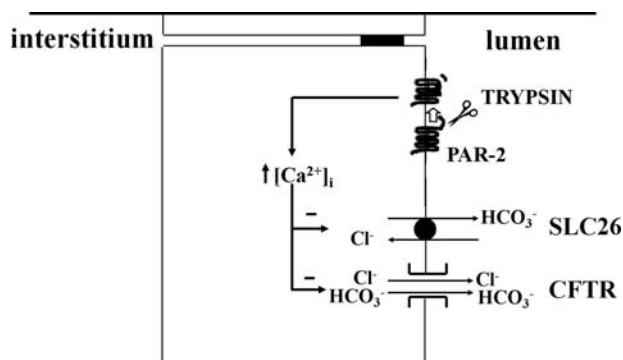


FIGURE 5. The effect of trypsin on pancreatic ductal epithelial cells. Trypsinogen can be prematurely activated to trypsin inside the ductal lumen during pancreatitis. Consequently, trypsin induces dose-dependent $[Ca^{2+}]_i$ elevation from intracellular Ca^{2+} stores via activation of protease-activated receptor 2. This causes reduction of ductal HCO_3^- secretion by ductal cells via inhibition of luminal Cl^-/HCO_3^- exchangers and CFTR Cl^- channel; –, inhibition.

mortality of patients with chronic pancreatitis.^{316,317} In fact, the detrimental effect of smoking on pancreatitis may be at least partly due to alteration of pancreatic ductal function. An interesting study by Kadiyala et al³¹⁸ showed that HCO_3^- concentration of pancreatic fluid in smokers is significantly reduced (indicating decreased ductal function) compared with non-smokers. The consequent reduction of ductal luminal pH, fluid secretion, and increased pancreatic protein secretion lead to protein plug and stone formation.

Pancreatic function tests can be used to determine the reserve of the pancreas in the clinical setting, although quite often these are not readily available. Most tests (eg, fecal elastase, fecal fat quantification, Lundh, and the ^{13}C -mixed triglycerides breath tests) are used to evaluate the acinar reserve, whereas the secretin test is used to measure secretory volume and HCO_3^- flow.³¹⁹ Nevertheless, pancreatic function/reserve can also be roughly estimated based on imaging such as endoscopic retrograde pancreatography, computed tomography, endoscopic ultrasound, and secretin-enhanced magnetic resonance cholangiopancreatography (MRCP).³²⁰ Magnetic resonance cholangiopancreatography has a fundamental role in visualization of the hepatobiliary ductal system. The technique is commonly performed with heavily T2-weighted sequences to highlight static or slow-moving fluids.³²¹ In the last 15 to 20 years, stimulation of pancreatic and biliary secretion by intravenous administration of secretin has been utilized for enhancing MRCP.³²² This allows the radiologist to better visualize the ductal system compared with MRCP without secretin stimulation. The diagnostic potential of secretin-enhanced MRCP is equivalent to the more conventional endoscopic retrograde cholangiopancreatography.³²³ Secretin-enhanced MRCP allows us to noninvasively estimate the pancreatic secretory volume and thus can be useful for quantification of exocrine function.³²⁴ The advantage of secretin-enhanced MRCP versus endoscopic retrograde cholangiopancreatography is that the former has no risk of iatrogenic acute pancreatitis. Indications of this technique include the detection and characterization of pancreatic ductal anomalies and strictures, evaluation of the integrity of the pancreatic duct, characterization of any communication between the pancreatic duct and pseudocysts/pancreatic fistulas, and the assessment of pancreatic function and sphincter of Oddi dysfunction.³²¹ Although reduction of HCO_3^- secretion is 1 of the earliest features of chronic pancreatitis, the availability and price of secretin limit the use of this gastrointestinal hormone for functional and imaging tests.

Autoimmune pancreatitis is a rare disease of the pancreas that can be cured by administration of steroids.^{325,326} Ito et al³²⁷ found that 11 of 12 patients with autoimmune pancreatitis showed reduction in pancreatic volume and amylase output, whereas reduction in HCO_3^- secretion was observed in only 5 of 12 patients. An elegant study by Ko et al³²⁸ confirmed the role of pancreatic ducts in this unique form of pancreatitis. They showed that mislocalized CFTR inside the cytoplasm of duct cells was a characteristic feature of autoimmune pancreatitis, which was associated with a reduction of secretin-induced pancreatic HCO_3^- secretion. Because CFTR mislocalization was also detected in a small number of patients with alcoholic pancreatitis and with obstructive pancreatitis, this may be the case in all forms of chronic pancreatitis (although this needs to be confirmed on a larger population of patients). Treatment of autoimmune pancreatitis with corticosteroids not only corrected the localization of CFTR to the apical membrane, but also reversed the impaired HCO_3^- secretion. Ko et al³²⁹ have also shown that pancreatic ductal dysfunction in alcoholic chronic pancreatitis does not recover within a year without active anti-inflammatory therapy.

The importance of intracellular Ca^{2+} signaling in the pathogenesis of chronic pancreatitis is highlighted by the fact that polymorphisms in the Ca^{2+} -sensing receptor gene increase the risk of the disease.^{330,331} The role of CFTR in the pathogenesis of idiopathic pancreatitis has also been supported by results of genetic studies.^{332,333} The first articles on CFTR mutations linked to a higher frequency of the disease were published in the *New England Journal of Medicine*,^{334,335} but these findings have been confirmed by numerous other investigators. Although 2 copies of severe CFTR mutations lead to CF, combinations of a severe CFTR mutation and a mild CFTR mutation lead to atypical CF and high pancreatitis risk, a severe CFTR mutation combined with pancreas divisum or SPINK1 mutation can result in increased susceptibility to sporadic chronic pancreatitis.³³⁶ Furthermore, it has recently been demonstrated that functional CFTR variants with impaired HCO_3^- (but not Cl^-) permeability increase the risk of pancreatitis but not for CF.^{38,337}

Diabetes Mellitus

There is close interaction of the exocrine and endocrine pancreatic parenchyma,³³⁸ so it is not surprising that ductal function is affected in diabetes mellitus, and vice versa, alteration of ductal function can also contribute to the development of diabetes. In fact, it is well known that a number of pancreatic diseases (CF, pancreatitis, and pancreatic adenocarcinoma) can cause diabetes, which always has to be kept in mind.

Many animal and human studies have demonstrated reduced pancreatic exocrine secretion (including total secretory volume pancreatic enzyme activity and HCO_3^- concentration) in both insulin-dependent and non-insulin-dependent diabetes mellitus, but the clinical relevance of these findings remains uncertain.^{338,339} Secretin-stimulated pancreatic secretion including volume and HCO_3^- output was decreased in patients with insulin-dependent^{340,341} and non-insulin-dependent diabetes mellitus³⁴² when compared with control subjects. The prevalence of exocrine insufficiency (which is usually mild to moderate, without clinical signs of exocrine pancreatic insufficiency) is somewhat lower in non-insulin-dependent (30%–50%) versus insulin-dependent (50%) diabetes mellitus,³³⁹ although in certain subgroups of patients, this rate may be much higher.³⁴³ Exocrine insufficiency seems to be correlated to the early onset of endocrine failure, long-lasting diabetes mellitus, and low body mass index levels.³⁴⁴

So how is pancreatic ductal secretion affected in diabetes mellitus? Importantly, diabetes inevitably causes imbalance of islet cell hormone levels. Given the stimulatory effect of

endogenous insulin on ductal secretion, one would expect to find decreased rates of secretion due to abnormal insulin secretion and/or insulin resistance. Indeed, this is actually the case, but not in all patients, so the involvement of other factors is likely. Nevertheless, insulin may be a vital trophic factor for not only acinar, but also for ductal cells. The reduced ductal secretion may be partly due to morphological alterations of the pancreas in patients with diabetes, such as smaller tissue size (atrophy), especially in insulin-dependent cases.³⁴⁵ Furthermore, duct morphology on endoscopic retrograde cholangiopancreatography in patients with diabetes is similar to the changes observed in chronic pancreatitis.^{346,347} Autoantibodies (eg, against carbonic anhydrase) found in patients with insulin-dependent diabetes may damage the ducts and cause compromised cellular function. Because ductal secretion is regulated by the nervous system, one cannot exclude the detrimental effects of autonomic neuropathy in diabetes mellitus.³³⁹ In addition, high glucose concentrations were found to inhibit rat pancreatic interlobular ductal HCO_3^- secretion.³⁴⁸ To explain the mechanism of reduced secretion, the authors hypothesized that glucose enters the cell via the sodium-dependent glucose transporter, and the consequent increase in $[\text{Na}^+]_i$ causes depolarization, which will reduce the driving force for HCO_3^- secretion.³⁴⁸ Incubation of ducts isolated from streptozotocin-treated diabetic animals in normal glucose solutions reversed the defect in secretion. Another possible explanation of decreased ductal secretion was proposed by Hootman et al.³⁴⁹ The increases in cyclic AMP levels evoked by exposure to secretin were not significantly different in pancreatic ducts isolated from healthy and diabetic guinea pigs, nor were levels of CFTR or Na^+/K^+ -ATPase expression.³⁴⁹ However, Na^+/K^+ -ATPase activity in pancreatic ducts isolated from diabetic animals was markedly decreased, suggesting a change in the enzyme's catalytic properties in the diabetic tissues. Notably, in a vicious cycle, reduced ductal secretion can also lead to a further impairment of exocrine pancreatic function in diabetic conditions.

CONCLUSIONS AND FUTURE DIRECTIONS

It is without a doubt that the most important ion in terms of pancreatic ductal secretion is HCO_3^- . The majority of pancreatic fluid originates from duct cells; secretion is mediated via paracellular and transcellular pathways. We have learned much in recent decades about the mechanism and regulation of pancreatic ductal HCO_3^- and fluid secretion, yet our knowledge is still incomplete. Without a doubt, secretion is mediated by a complex interaction of different transporters. The most important transporters involved in ductal secretion have been identified; however, it is still unclear how the luminal 140 mM HCO_3^- concentration is reached during stimulated conditions in humans. There is ample evidence that CFTR has a central role in pancreatic ductal HCO_3^- and fluid secretion. Cystic fibrosis transmembrane conductance regulator not only conducts anions such as Cl^- and HCO_3^- , but it also interacts with numerous transporters involved in secretion. Studying the distribution and interaction of transporters in/within different segments of the ductal tree may shed further light on the exact mechanism of HCO_3^- secretion.

The localization and function of CFTR are also affected by severe diseases such as CF and pancreatitis. The resulting alterations of pancreatic HCO_3^- and fluid secretion are characteristic features of these diseases. Cystic fibrosis causes a marked reduction in ductal secretion, eventually leading to the destruction of the gland. The utilization of newly developed pig and ferret CF models (which better mimic the human disease compared with rodents) will help us in better understanding the disease.

However, for most researchers, these models remain inaccessible. Possible therapeutic targets in restoring defective ductal secretion in CF include the Ca^{2+} -activated Cl^- channels and the restoration of CFTR activity by correctors, potentiators, and gene transfer. Pancreatitis-inducing agents (such as bile acids and ethanol) have a dose-dependent effect on ductal secretion; low concentrations stimulate secretion, whereas high concentrations inhibit secretion. The modulation of ductal secretion may have beneficial effects in the prevention and/or treatment of pancreatitis that is thought to be primarily a disease of the acinar cells.

Despite some marked differences between pancreatic acinar and ductal cells, they both seem to be damaged by toxic factors in a similar way. Therefore, therapeutic options are likely to help acinar and ductal cells. In fact, we have quite a few therapeutic targets such as intracellular ATP depletion and toxic Ca^{2+} signaling that need to be investigated further and taken from the bench to bedside. For example, the latter is already on its way, by testing the effect of the " Ca^{2+} antagonist" MgSO_4 in the prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis.³⁵⁰ More such randomized clinical trials are desperately needed in evaluating treatments of pancreatic diseases.

Notably, our current understanding of the physiology and pathophysiology of pancreatic secretion mainly comes from animal studies. As mentioned previously, marked differences exist in the secretory and disease mechanisms of various species. Therefore, the results obtained from animal experiments need to be interpreted with caution and cannot necessarily be directly adapted to the human situation. This is especially true when we take a look at the low number of therapeutic interventions that have made it to clinical practice. Hopefully, the situation will improve by finding/using better disease models.

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