7 Physiology of duct cell secretion

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Introduction

The pancreas secretes digestive enzymes and a fluid rich in HCO_3^- and poor in Cl^-. The digestive enzymes are synthesized and secreted by the acinar cells, which also secrete a small volume of an isotonic NaCl-rich fluid. The pancreatic duct secretes the bulk of the HCO_3^--rich fluid [1,2]. While exocytotic enzyme secretion by acinar cells has been studied extensively [3], the molecular mechanism of fluid secretion by the duct is only partially understood [1]. This is despite the fact that we now know that impaired ductal secretion leads to destruction of the pancreas, as occurs in cystic fibrosis [4,5], and may contribute to other diseases of the pancreas including alcohol-associated chronic pancreatitis [6]. Hence beside its intrinsic interest, understanding the mechanisms of pancreatic ductal fluid and electrolyte secretion has an immediate link to a better understanding of common pancreatic diseases.

The ductal tree provides a structural framework for acinar and endocrine tissues, secretes fluid that acts as a vehicle for the transport of digestive enzymes, and secretes HCO_3^- that neutralizes gastric acid and provides an optimum pH environment for digestive enzymes in the duodenum [2,7]. An overlooked function of HCO_3^- secretion is that HCO_3^- is a chaotropic ion that is important for the solubilization of macromolecules in order to prevent the aggregation of digestive enzymes and mucins. This chapter focuses on the molecular mechanisms of fluid and HCO_3^- secretion and their regulation and attempts to explain how the duct secretes HCO_3^- at a concentration more than five times higher than that found in plasma.

Structure of the ductal tree

The pancreas develops from the ventral and dorsal surfaces of the primitive foregut, and the two parts later fuse to form a complex endocrine–exocrine organ. Acinar and islet cells are derived from the ductal bud, which has stem cell-like properties. Even after completion of morphogenesis, duct cells retain some proliferative capacity in order to generate new duct and islet cells [8].

In humans and most other mammals, duct cells comprise about 10% of the number of cells and 5% of the total mass of the pancreatic gland [9]. The duct endings are connected to a group of acinar cells to form the acini (Fig. 7.1). The acinar cells are in contact with centroacinar cells, which have several ductal characteristics and are regarded as the terminal cells of the ductal tree. The contents of the acini empty into the intercalated, intralobular, and finally the interlobular ducts (Fig. 7.2). In humans, the interlobular ducts join to form the main pancreatic duct, which shares a duodenal opening with the common bile duct at the ampulla of Vater. However, in rodents a number of interlobular ducts open directly into the common pancreaticobiliary duct without forming a main duct.

In the human pancreas, the intercalated and the small intralobular ducts are the major sites of HCO_3^- secretion. In the mouse and rat, the interlobular duct secretes the bulk of the fluid and HCO_3^- [11]. The HCO_3^--secreting portion of these ducts is lined by the principal cells, which share common characteristics. They contain a relatively small amount of rough endoplasmic reticulum, Golgi complexes, and secretory vesicles, but are rich in mitochondria in order to satisfy the energy requirements of active HCO_3^- secretion. The luminal (apical) membrane of the principal cell possesses microvilli. The lateral membranes are interdigitated and linked by tight and adherent junctions and by desmosomes. In the larger ducts the principal cells become columnar and the duct contains goblet cells, which are specialized in secreting mucins.

Composition of pancreatic juice

The human pancreas secretes 1–2 L of pancreatic juice per day in response to physiologic stimuli, mainly secretin and vagal output. The pancreatic juice is a clear, alkaline, isotonic fluid. The human pancreatic duct can secrete a fluid containing 120–140 mmol/L HCO_3^- [12]; however, in the rat the HCO_3^- concentration is about 70 mmol/L [13]. The HCO_3^- content in the juice increases with increased flow rate. Peak HCO_3^- content is reached at 30–50% of maximal flow. The reciprocal Cl^- absorption and HCO_3^- secretion results in isotonic osmolality at all flow rates (Fig. 7.3) [13–15]. The cation composition of the juice is nearly constant (140 mmol/L Na^+ and 10–15 mmol/L K^+) regardless of flow rate. Human pancreatic juice also contains 1–2 mmol/L Ca^{2+} and a small amount of Mg^{2+}, Zn^{2+}, PO_4^{3-}, and SO_4^{2-}.

Regulation of pancreatic secretion

The principle involved in the control of pancreatic fluid and electrolyte secretion is that of the common neurohumoral control

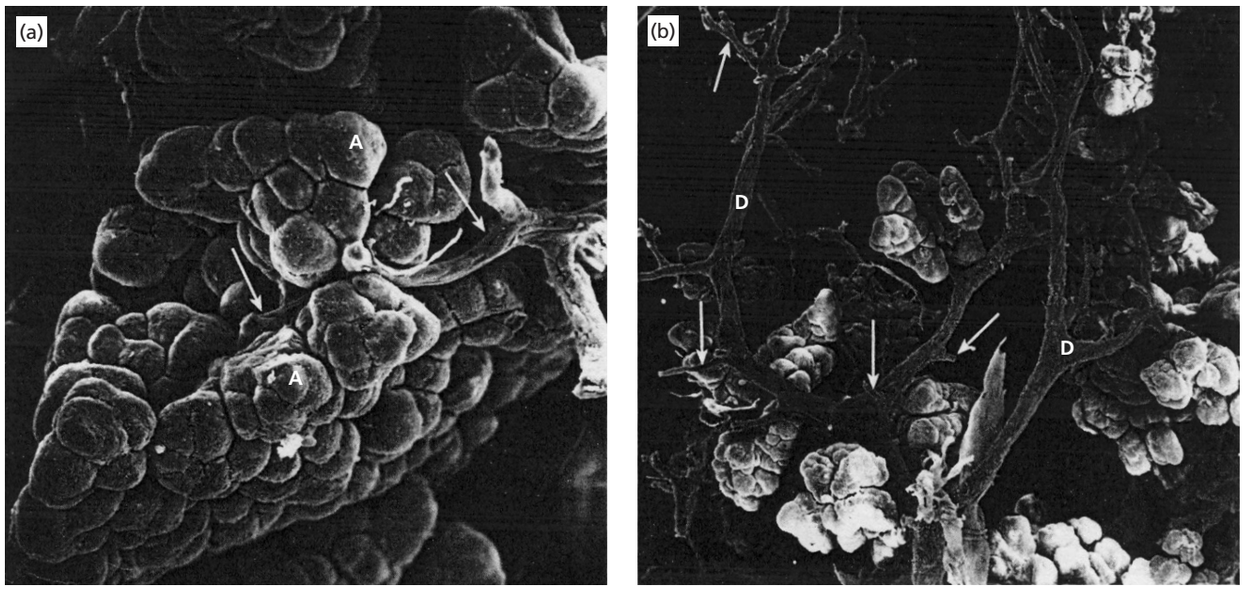

Figure 7.1 (a) Scanning electron microscopic view of rat pancreatic lobules. Arrows indicate a long intercalated duct. (b) Scanning electron microscopic view of rat pancreatic ducts after removal of most acini by ultrasonic vibration. Arrows indicate intercalated ducts. (From ref. 10 with permission.)

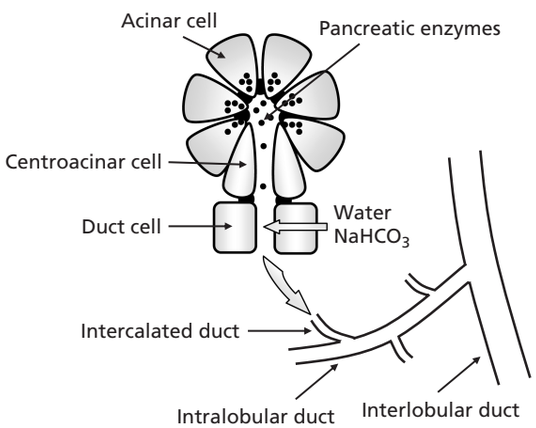

Figure 7.2 Schematic diagram of the pancreatic exocrine system. The endings of the terminal duct are connected to a group of acinar cells to form an acinus. The contents of the acini empty into intercalated ducts, intralobular ducts and, in turn, small and large interlobular ducts. The pancreatic duct secretes the bulk of the fluid in the pancreatic juice that is rich in HCO_3^-.

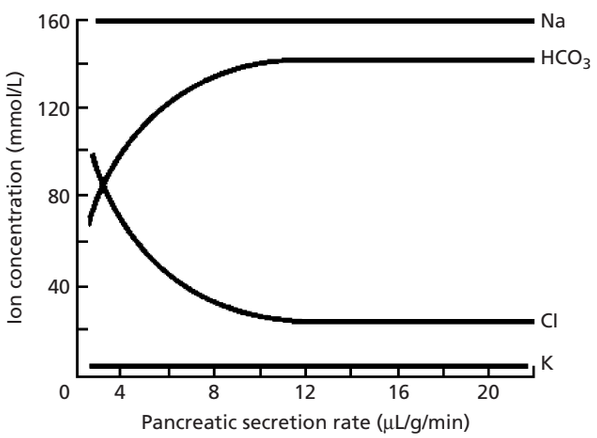

Figure 7.3 Composition of pancreatic juice at different flow rates in the cat in response to secretin stimulation. The reciprocal Cl^- absorption and HCO_3^- secretion results in juice with isotonic osmolality regardless of flow rate. (From ref. 16 with permission.)

of gastrointestinal secretion as proposed over 100 years ago [17,18], and includes vagal and secretin stimulation. However, recent findings indicate that the regulation of pancreatic secretion is more complex, with numerous stimulatory and inhibitory inputs [19]. Another consideration when studying pancreatic secretion is the highly species-specific pattern of secretion. In this chapter, we emphasize the physiology of the human pancreas, and list information obtained with the guinea-pig pancreas, the secretion of which most resembles that of the human. When informative, data obtained with genetically manipulated mice are also discussed. Additional information can be found in references 7 and 15.

Interdigestive secretion

In dogs and possibly in humans, interdigestive (resting) pancreatic HCO_3^- and digestive enzyme secretion is less than 2% and 10% of the maximum, respectively [20]. Ductal HCO_3^-

salvage mechanisms contribute to the low basal HCO_3^- output [21]. During the interdigestive period, transient bursts of secretion occur every 1–2 hours. This coincides with the cyclical motor activity of the upper gastrointestinal tract (interdigestive migrating motor complex, IMMC). Gastric and biliary secretion, and blood levels of motilin and pancreatic polypeptide (PP), also increase along with the IMMC. The IMMC is generated by circulating motilin and by cholinergic stimulation [22]. The precise physiologic significance of the IMMC is uncertain.

Response to a meal

Pancreatic HCO_3^- and enzyme secretion are increased in response to a meal. Control of pancreatic secretion is divided into the cephalic, gastric, and intestinal phases. Secretion by acinar cells is controlled by the cephalic and gastric inputs. For example, sham feeding with visual, olfactory, and gustatory stimuli increases enzyme secretion by up to 50% of maximum in humans [23]. Gastric distension with a balloon also increases enzyme output [24]. However, both stimuli have little effect on ductal HCO_3^- and fluid secretion.

The intestinal phase is the most important phase for meal-induced pancreatic secretion and commences with the passage of chyme into the proximal duodenum. Secretin, secreted by cells in the upper duodenum, and an enteropancreatic vago-vagal reflex are the two principal mechanisms that induce ductal fluid and HCO_3^- secretion. Anti-secretin antibodies block about 80% of postprandial HCO_3^- output [25], highlighting the central role of secretin in stimulating HCO_3^- secretion. However, exogenous application of secretin that elevates plasma concentration to that observed in the postprandial state does not evoke ductal secretion to the extent observed in the postprandial secretion [26]. This suggests that other factors, such as cholecystokinin (CCK) and vagal stimulation, contribute significantly and synergize with secretin to stimulate ductal fluid and HCO_3^- secretion. The decrease in secretin-evoked HCO_3^- secretion by atropine and vagal blockade indicates that vagal cholinergic input is important for postprandial HCO_3^- secretion [27]. The exogenous in vivo application of CCK potentiates secretin-stimulated fluid and HCO_3^- secretion [28].

Mode of action

Various chemical mediators affect pancreatic fluid and HCO_3^- secretion. Candidate mediators that stimulate, inhibit, or have variable effects on pancreatic fluid and HCO_3^- secretion are listed in Tables 7.1–7.3. They are involved in both the humoral and the neuronal control of pancreatic secretion during physiologic and pathologic states.

The pancreatic duct expresses receptors for a battery of hormones and neurotransmitters that reach the duct via the bloodstream or by release from nerve terminals to varicosities. Hormones (endocrine) like secretin -are released by distant organs and require high-affinity receptors. A large number of other humoral agents are released by the pancreas to modulate its secretion. Cells in the islets of Langerhan release insulin, somatostatin, and several other peptide hormones that affect ductal and acinar secretion. These agents are present at relatively high concentration and reach their target through the insuloacinar portal system [29]. Other agonists, including purines, prostaglandins, and activated trypsin, are released by either acinar or duct cells and regulate ductal function in numerous physiologic and pathologic states. Receptors for these agonists (autacoids, paracrine) are expressed in both the luminal and the basolateral membranes of the duct.

Both the extrinsic and the intrinsic nervous systems participate in the neuronal control of pancreatic secretion. Nerve fibers travel through the lamina propria of the intercalated, interlobular, and main ducts. The nerve terminals are located in close proximity to the duct basal membrane [30]. In this manner the neurotransmitters diffuse only a short distance, retain a high concentration at their effector sites, and produce a relatively rapid response. Some agonists, such as ATP, are released by both neuron and the pancreatic acinar and duct cells and activate the same class of receptors [31].

Endocrine and paracrine control of ductal secretion

Secretin

The entry of acidic chyme into the duodenum evokes the release of secretin from neuroendocrine cells in the duodenal mucosa. Numerous studies in animals and humans point to the principal role of secretin in postprandial fluid and HCO_3^- secretion. These include (i) a rise in plasma secretin after a meal [32], (ii) a linear relationship between the rise in plasma secretin and HCO_3^- output [33], and (iii) inhibition of postprandial pancreatic HCO_3^- secretion by serum anti-secretin antibodies [25]. Plasma secretin in response to a meal reaches only picomolar levels, which is sufficient to stimulate fluid and HCO_3^- secretion in all species. CCK and vagal stimulation further potentiate the secretin-stimulated secretion [27,28]. Intraduodenal pH below 4.5 is a prime stimulus of secretin release [32,34]. Other stimuli of secretin secretion are fatty acids and a high concentration of bile salts [35].

Cholecystokinin

The effect of CCK on ductal secretions varies between species. In humans, the infusion of CCK alone weakly stimulates fluid secretion but greatly potentiates the effects of secretin [2]. Recent studies have revealed that in humans the effects of CCK are mediated by stimulation of vagal afferent fibers and that the human pancreas does not express CCK-A receptors [36]. Endogenously released CCK is heterogeneous and consists of multiple forms, such as CCK58, CCK33, and CCK8, although CCK58 is likely the major form that stimulates vagal afferent fibers [37].

Purines

Purinergic receptors (P2Rs) are classified into metabotropic P2Y and ionotropic P2X receptors. The pancreatic duct

Table 7.1 Agonists that stimulate pancreatic fluid and HCO_3^- secretion.

Agent	Mode of action	Remark	Studied species
Secretin	Endocrine	Major stimulant of pancreatic HCO_3^- and fluid secretion Released by small intestine Increases cAMP in duct cells	Human, cat, dog, rat, guinea-pig, hamster, rabbit, pig
CCK	Endocrine/neuronal	Potentiates secretin-stimulated HCO_3^- secretion Released by small intestine Main action in human is stimulation of vagal afferent fibers	Human, cat, dog, rat, guinea-pig, hamster, rabbit, pig
Acetylcholine	Neuronal	Potentiates secretin-stimulated HCO_3^- secretion Neurotransmitter in vagal efferent fibers Increases $[Ca^{2+}]_i$ in duct cells	Human, cat, dog, rat, guinea-pig, hamster, rabbit, pig
VIP	Neuronal	Stimulates HCO_3^- and fluid secretion at high concentrations Neurotransmitter in vagal efferent fibers Increases cAMP in duct cells	Human, cat, dog, rat, pig
PHI	Neuronal	Weak stimulant of fluid secretion Colocalizes with VIP	Human, dog, pig
Bombesin/neuromedin/ GRP	Endocrine/neuronal	Stimulates HCO_3^- and fluid secretion in some species	Dog, rat, rabbit, hamster, guinea-pig, pig
Neurotensin	Endocrine	May stimulate HCO_3^- and fluid secretion in humans Released by distal small intestine	Human, dog, rat, pig
Insulin	Endocrine/paracrine	Exogenous insulin increases CCK-induced fluid secretion Released by the endocrine pancreas	Cat, rat
PAR2	Paracrine	Stimulates fluid secretion in rat Stimulates apical HCO_3^--secreting transporters in human and dog duct cell lines Basolateral PAR2 are stimulated by trypsin probably released during acute pancreatitis	Rat (human, dog)
NO	Neuronal/paracrine	NO stimulates pancreatic secretion Deletion of eNOS, but not iNOS and nNOS, inhibits pancreatic secretion in mice	Monkey, mouse, rat, pig
ANF/ANP	Endocrine/paracrine	Dose-dependent increases in fluid and HCO_3^- secretion Released by acinar and duct cells	Dog, rat, rabbit

ANF/ANP, atrial natriuretic factor/peptide; CCK, cholecystokinin; GRP, gastrin-releasing peptide; NO, nitric oxide; eNOS/iNOS/nNOS, endothelium/inducible/neuronal nitric oxide synthase; PAR2, protease-activated receptor 2; PHI, peptide histidine isoleucine; VIP, vasoactive intestinal peptide.

expresses multiple P2YRs and P2XRs, both at the apical and the basolateral membranes [38]. Stimulation of ductal P2Rs of several animals and of human ductal cell lines stimulates fluid secretion and activates membrane transporters that enhance HCO_3^- secretion [31,39,40]. However, stimulation of apical and basolateral P2Rs can differentially affect guinea-pig ductal secretion [41]. Several purines and pyrimidines found in the extracellular fluid (i.e., ATP, ADP, adenosine, UTP, and UDP) can activate P2Rs. Possible sources of purinergic ligands include release by nerve terminals at the basolateral space, release from zymogen granules of acinar cells, and efflux by ductal ATP transporters [31]. Although the purinergic system likely plays a role in the regulation of pancreatic ductal secretion under physiologic and pathologic states, its specific role in humans has not been demonstrated as yet.

Compounds secreted by the endocrine pancreas

The islets secrete multiple hormones, including insulin by β cells, glucagon by α cells, somatostatin by δ cells, and pancreatic polypeptide (PP) by PP cells. Exocrine cells are exposed to relatively high concentrations of these hormones [29]. Insulin increases fluid and enzyme secretion in the rat [42], and decreased ductal secretion is observed in patients with type 1 diabetes [43]. Somatostatin, PP, and glucagon have all been reported to inhibit pancreatic secretions in many species,

Table 7.2 Agonists that inhibit pancreatic fluid and HCO_3^- secretion.

Agent	Mode of action	Remark	Studied species
Somatostatin	Endocrine/paracrine	Inhibits secretin-stimulated secretion Released by endocrine pancreas May act indirectly on pancreatic duct cells	Human, cat, dog, rat, rabbit, pig
PP	Endocrine/paracrine	Inhibits HCO_3^- and fluid secretion in experimental animals Released by the endocrine pancreas	Cat, dog, rat, pig
PYY	Endocrine	Inhibits HCO_3^- and fluid secretion in experimental animals Released by the distal small intestine	Cat, dog
Glucagon	Endocrine/paracrine	Inhibits HCO_3^- and fluid secretion in experimental animals No inhibitory effect reported in humans Released by the endocrine pancreas	Human, cat, dog, rat
Pancreastatin	Endocrine/paracrine	Inhibits vagal-stimulated secretion Released by the pancreas and gastrointestinal tract	Rat
Vasopressin/ADH	Endocrine	Inhibits secretin-stimulated secretion Released by the posterior hypophysis	Human, dog
SP	Neuronal	Inhibits fluid secretion in isolated rat and guinea-pig duct Colocalizes with CGRP in sensory nerve endings Stimulates fluid secretion in dogs	Rat, dog
CGRP	Neuronal	Inhibits secretion, most likely through release of somatostatin in dogs Colocalizes with SP in sensory nerve endings	Dog
NPY	Neuronal	Inhibits fluid secretion but increases HCO_3^- secretion in pigs Colocalizes with catecholamines in sympathetic fibers	Rat, pig

ADH, antidiuretic hormone; CGRP, calcitonin gene-related peptide; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY; SP, substance P.

Table 7.3 Agonists with variable effects on pancreatic fluid and HCO_3^- secretion.

Agent	Mode of action	Remark	Studied species
Epinephrine/ norepinephrine	Neuronal	Stimulation of α-adrenergic receptors inhibits HCO_3^- secretion, stimulation of β-adrenergic receptor increases HCO_3^- secretion No significant effects of catecholamines in humans reported Released by sympathetic (splanchnic) nerve endings	Human, cat, dog, rat, rabbit, pig
Dopamine	Neuronal	Dopamine and D-, L-dopa stimulate HCO_3^- secretion in cats, dogs, and rats, but inhibit HCO_3^- secretion in rabbits No significant effects in humans	Human, cat, dog, rat, rabbit
Histamine	Neuronal/paracrine	Stimulation of H_1 and H_2 receptors increases HCO_3^- secretion, probably secondary to increased blood flow In rabbits, H_2 stimulation inhibits HCO_3^- secretion	Dog, rat, rabbit
5HT	Paracrine/neuronal	Activation of $5HT_2$ and $5HT_3$ receptors in intestinal vagal afferent fibers stimulates pancreatic secretion Synergistic interaction with CCK Stimulation of $5HT_3$ receptor in isolated guinea-pig ducts inhibits HCO_3^- secretion	Rabbit, guinea-pig
Purines (ATP)	Neuronal/paracrine	Adenosine and ATP potentiate secretin-stimulated HCO_3^- secretion in dogs Basolateral ATP stimulates but apical ATP inhibits fluid secretion in isolated guinea-pig ducts Released by vagal nerve endings, acinar cell granules, and miscellaneous sources	Dog, guinea-pig
Prostaglandins	Paracrine	PGE_2 slightly increases spontaneous HCO_3^- secretion in humans but has no effect on secretin-stimulated secretion Effects vary according to species and prostaglandin	Human, cat, dog, rat

CCK, cholecystokinin; 5HT, 5-hydroxytryptamine; PGE_2, prostaglandin E_2.

including humans (see Table 7.2). The effect of somatostatin is indirect, and involves inhibition of the release of humoral mediators and of the intrapancreatic nervous system [44]. Although somatostatin is a major negative regulator of pancreatic secretion, its exact physiologic role in pancreatic secretion remains to be elucidated.

Other humoral mediators

Additional mediators that affect pancreatic secretion are listed in Tables 7.1–7.3. Peptides belonging to the gastrin-releasing family (GRP, bombesin) are secreted by the gastrointestinal tract and the intrapancreatic nervous system [7]. In pigs, GRP evokes copious HCO_3^- secretion by stimulating secretin release [45]. Neurotensin is another stimulatory agent that is released predominantly by endocrine cells in the distal part of the small intestine. In humans neurotensin is increased in response to a meal and potentiates HCO_3^- secretion stimulated by other secretagogues [46]. Recent work suggests that serotonin (5-hydroxytryptamine, 5HT) may stimulate postprandial pancreatic secretion. Activation of $5HT_2$ and $5HT_3$ receptors in vagal afferent fibers by luminal food or by mechanical distension of the intestine stimulates pancreatic secretion in the rat [47]. On the other hand, 5HT acting on $5HT_3$ receptors in isolated guinea-pig ducts inhibits secretin-stimulated fluid secretion [48].

Peptide YY (PYY) is released by the ileum and colon, and inhibits pancreatic secretion [49] by an unknown mechanism. It has been suggested that PYY inhibits CCK release, stimulates inhibitory adrenergic fibers, or acts directly on pancreatic PP receptors [15].

Neuronal control

Pancreatic secretion is controlled by the enteric nervous system, which comprises a gut–brain axis and an intrapancreatic system. The intrapancreatic system is composed of an interconnecting plexus of ganglia and postganglionic fibers lying in the intralobular connective tissues, blood vessels, and occasionally in the neuronal trunk [50]. It is supplied by preganglionic parasympathetic (vagal) fibers, postganglionic sympathetic (splanchnic) fibers, and possibly other fibers that emanate from the gut wall.

Parasympathetic, vagal, and cholinergic network

The terms "parasympathetic," "vagal," and "cholinergic" are frequently considered together with reference to pancreatic function, because the vagal output provides the cholinergic regulation of pancreatic secretion via parasympathetic fibers. The parasympathetic nerve terminals contain additional neurotransmitters, such as vasoactive intestinal peptide (VIP) and ATP [31,50]. The intrapancreatic cholinergic neurons have intrinsic tone, which remains active after extrinsic denervation. Hence, caution must be exercised when interpreting the effects of vagal stimulation and cholinergic agents in tracing the origin of any observed effect.

The effect of vagal stimulation on pancreatic fluid secretion shows a species-specific pattern and is highly variable. Vagal stimulation in the pig and guinea-pig causes the secretion of a HCO_3^--rich fluid by releasing VIP from nerve terminals [50]. In humans, the vagovagal reflex and intrapancreatic cholinergic fibers enhance postprandial ductal secretion by potentiating the effect of secretin. Food content and/or mechanical distension of the intestine stimulate vagal afferent fibers. CCK and 5HT can also stimulate vagal afferent fibers [47]. Recent findings have revealed that the enteropancreatic vagovagal reflex in the central nervous system involves the lateral hypothalamic nucleus, paraventricular nucleus, and lateral parabrachial nucleus [51].

Sympathetic, splanchnic, aminergic network

Most of the aminergic neurons in the pancreas represent postganglionic sympathetic fibers of the splanchnic nerve whose cell bodies lie in the celiac ganglion. However, 10% of aminergic fibers remain after surgical sympathectomy in the rat, indicating an alternative origin. Like the vagal fibers, stimulation of the splanchnic nerve releases multiple neurotransmitters that include catecholamines, neuropeptide Y (NPY), and galanin [52,53]. Most of the aminergic fibers innervate blood vessels, and only a few are found in the vicinity of acinar and duct cells. Splanchnic stimulation inhibits pancreatic secretion, mainly due to reduced blood flow. However, in isolated ducts direct stimulation of β-adrenergic receptors evokes HCO_3^- secretion [54], although its physiologic role is uncertain.

Peptidergic and other neurotransmitters

VIP and peptide histidine isoleucine (PHI) are colocalized in the same neurons and are released by vagal stimulation [55]. Ample evidence shows that VIP stimulates ductal HCO_3^- secretion, which may play a physiologic role in several species, including humans [52]. Exogenous PHI stimulates HCO_3^- secretion in several species. NPY is localized in the postganglionic sympathetic fibers together with catecholamine and controls pancreatic blood flow to induce vasoconstriction. Hence, in general, NPY is regarded as an inhibitor of pancreatic secretion. Substance P and calcitonin gene-related peptide (CGRP) are colocalized in the same neurons and act as inhibitory neurotransmitters [52].

Mechanism of ductal fluid and electrolyte secretion

The two vital and coupled functions of the pancreatic duct are fluid secretion and the reciprocal Cl^- absorption and HCO_3^- secretion. The pancreatic duct is unique among absorbing and secretory epithelia in that it does not express the epithelial Na^+ channel (ENaC) and thus does not absorb Na^+. In fact, the pancreatic duct secretes Na^+, which passes paracellularly [1,2]. The duct could also secrete Cl^-, which is mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel [1,5], to drive fluid secretion. The pancreatic duct then absorbs Cl^- and secretes HCO_3^-. A unique feature of the pancreatic duct in most species, including

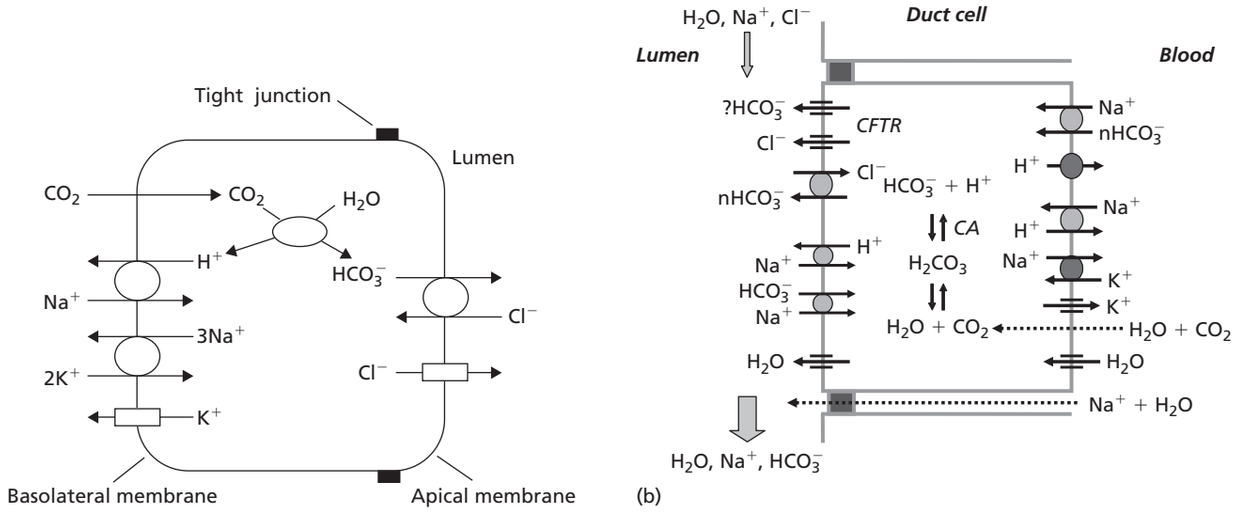

Figure 7.4 Transporters mediating HCO_3^- secretion by pancreatic duct cells. (a) The model proposed to account for ductal HCO_3^- secretion. (From ref. 1 with permission.) (b) Localization of membrane transporters associated with the vectorial transport of HCO_3^- listed and discussed in this chapter.

humans, is the secretion of a fluid containing as much as 140 mmol/L HCO_3^-.

In the late twentieth century, a model of pancreatic HCO_3^- secretion was proposed by Argent and Case, as shown in Fig. 7.4a [1,2]. However, it is difficult to reconcile this model with the properties of ductal secretion. First, the exchange of Cl^- for HCO_3^- cannot promote fluid secretion, which requires net salt transport. Second, this model can generate a fluid containing at most 70 mmol/L HCO_3^-, much lower than the 140 mmol/L found in human and guinea-pig pancreatic juice. Moreover, experiments with microperfused intralobular and main ducts confirmed the presence of a basolateral Na^+/H^+ exchanger (NHE) and a luminal Cl^-/HCO_3^- exchanger, but also revealed the presence of basolateral Na^+/HCO_3^- cotransporter (NBC) and Cl^-/HCO_3^- exchanger and, surprisingly, luminal NHE and NBC [56].

Transporters in duct cells

In the early 1990s, the identity of the acid–base transporters at the basolateral and luminal membranes of the pancreatic duct began to be characterized [56]. The first major discovery was the finding of the luminal HCO_3^--absorbing NHE [21,56] and NBC3 [57]. This suggested that during the resting state the duct absorbs and scavenges HCO_3^-. Another important discovery was finding a basolateral NBC [56], which is critical for pancreatic HCO_3^- secretion [58]. Even more important was the finding that several CFTR mutants associated with pancreatic insufficiency retained substantial Cl^- channel activity but lost their ability to support HCO_3^- secretion [59]. These findings drew considerable attention to the problem of HCO_3^- secretion in cystic fibrosis and subsequently in pancreatitis. Recent studies have revealed the essential role of the SLC26 family of Cl^-/HCO_3^- transporters in pancreatic HCO_3^- secretion [60]. A comprehensive review of this topic is provided in Ref. 1. Here, we summarize the molecular mechanism of ductal fluid and electrolyte secretion, with an emphasis on the tightly coupled Cl^- absorption and HCO_3^- secretion. Membrane localizations of key transporters and their transport mode are illustrated in Fig. 7.4b.

Na^+/H^+ exchangers and H^+-ATPase

The NHEs are electroneutral $1Na^+/1H^+$ exchangers. The housekeeping NHE1 is localized at the basolateral membrane [21] and guards against cytoplasmic acidification. It has a secondary role in HCO_3^- secretion since inhibition of NHE1 minimally affects HCO_3^- secretion. The luminal NHEs comprise NHE2 and NHE3 [21] and, interestingly, are regulated by CFTR [61]. A V-type H^+-ATPase has been proposed as the mediator of HCO_3^- uptake at the basolateral membrane [62]. However, although inhibition of H^+-ATPase diminishes HCO_3^- secretion in pigs [62], no effect was found in guinea-pigs [63].

Na^+/HCO_3^- cotransporters

In all species most HCO_3^- uptake across the basolateral membrane during stimulated HCO_3^- secretion is mediated by the electrogenic pancreatic isoform, pNBC. pNBC mRNA is abundant in pancreatic acinar and duct cells [64]. A robust Na^+/HCO_3^- cotransport activity was found in the basolateral membrane of rat pancreatic acinar and duct cells [65] and guinea-pig duct [66]. The stoichiometry of pNBC depends on the cell in which it is expressed, and can be altered by phosphorylation of Ser1026 by protein kinase A (PKA) [67]. pNBC behaves as

a $1Na^+/2HCO_3^-$ cotransporter when expressed in a pancreatic ductal cell line. Although the stoichiometry of transport was not measured directly in native pancreatic ducts, it must be $1Na^+/2HCO_3^-$ in the stimulated state since it mediates HCO_3^- influx across the basolateral membrane [1], which at a membrane potential of −60 mV is possible only with a $1Na^+/2HCO_3^-$ stoichiometry.

The luminal NBC is the electroneutral NBC3 [57]. The finding of Na^+- and HCO_3^--absorbing mechanisms at the luminal membrane of the duct was unexpected. However, NHE3 and NBC3 are regulated by CFTR and stimulation of CFTR with PKA leads to inhibition of these transporters [61,68]. Therefore, it seems that NHE3 and NBC3 are part of the HCO_3^--regulating complex of the duct, and salvage HCO_3^- at rest in order to maintain acidified pancreatic juice [68]. In this model, when the duct absorbs HCO_3^- at rest, the stoichiometry of pNBC is likely $1Na^+/3HCO_3^-$. With a membrane potential of −60 mV, pNBC extrudes HCO_3^- across the basolateral membrane that is absorbed by the luminal NHE3 and NBC3. It remains to be determined if indeed the stoichiometry of pNBC in native ducts switches from $1Na^+/3HCO_3^-$ to $1Na^+/2HCO_3^-$ as the ducts switch from an HCO_3^--absorbing to an HCO_3^--secreting mode.

Cl^-/HCO_3^- exchangers and the SLC26 transporters

The basolateral membrane anion exchanger (AE) is the housekeeping AE2 (SLC4A2). AE2 protects the cells against an alkali load and does not play a major role in HCO_3^- secretion or absorption. The molecular identity and function of the luminal membrane AE remained a mystery for a long time. A breakthrough was made with the discovery that the protein known as DRA (downregulated in adenoma) is mutated in congenital Cl^- diarrhea and is expressed at high levels at the luminal membrane of the colon and functions as a Cl^- transporter [69]; it was subsequently shown to function as a Cl^-/HCO_3^- exchanger [70]. DRA belongs to the family of SLC26 transporters: it is designated SLC26A3 and is expressed in several epithelia. Notably, SLC26A3 functions as an electrogenic $2Cl^-/1HCO_3^-$ exchanger [60,71]. SLC26A6 was originally identified in a search for novel SLC26 transporters [72], and as the oxalate transporter in the renal proximal tubule [73]. SLC26A6 and its two splice variants are ubiquitously expressed, with high levels at the luminal membrane of the pancreatic duct [72]. SLC26A6 functions as a $2HCO_3^-/1Cl^-$ exchanger [71,73]. SLC26A2 and SCL26A11 are ubiquitous and their mRNA is expressed in the pancreatic ducts (M.G. Lee & S. Muallem, unpublished results). The ductal function of SLC26A2 and SLC26A11 is not known at present. The pancreatic duct and acinar cells express multiple SLC26 transporters and undoubtedly their role in pancreatic HCO_3^- secretion and function will be revealed in coming years.

Cystic fibrosis transmembrane conductance regulator

CFTR is the central regulator and mediator of fluid and electrolyte transport by the pancreatic duct. Indeed, CFTR regulates many of the transporters involved in these processes. This is best exemplified in the aberrant fluid and electrolyte transport and pancreatic insufficiency seen in cystic fibrosis. 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 (PSD-95/disks large/ZO-1) ligand that binds to the scaffold NHERF/EBP50 [74]. Subsequently, CFTR was found to interact with several scaffolds, SNARE proteins like syntaxin 1A, and with AKAPs, kinases and phosphatases [75]. In addition, CFTR interacts both directly and indirectly with several ion transporters and regulates their activity. Functional, and in some cases biochemical, interactions with CFTR were reported with ENaC (not expressed in the pancreatic duct), the outwardly rectifying and Ca^{2+}-activated Cl^- channels, ROMK2 and KvLQT1 K^+ channels and aquaporin AQP3.

Because CFTR functions as a Cl^- channel with some permeability to HCO_3^- [76] and because the absence of CFTR activity in cystic fibrosis results in inhibition of HCO_3^- secretion, it was assumed that CFTR mediates the tightly coupled Cl^- absorption and HCO_3^- secretion. However, Cl^- and HCO_3^- permeability are segregated in several CFTR mutations associated with cystic fibrosis [59]. Furthermore, the HCO_3^- permeability of CFTR is dynamically regulated by extracellular Cl^- [71,77]. CFTR does not transport HCO_3^- in the presence of physiologic Cl^- in the duct lumen. Only when luminal Cl^- is reduced to about 20 mmol/L (as in the stimulated duct) does a switch in CFTR Cl^-/HCO_3^- permeability occur to increase the HCO_3^- permeability of CFTR to an extent that CFTR can participate in HCO_3^- secretion [71].

Since Cl^- absorption and HCO_3^- secretion requires CFTR, but CFTR cannot transport HCO_3^- in the proximal duct, the question is how CFTR regulates HCO_3^- secretion. An answer was provided by the discovery of the interaction and reciprocal regulation of CFTR with the ductal HCO_3^- secretory mechanisms. HCO_3^- transport by the pancreatic duct is mediated by multiple transporters that are assembled into complexes by adaptor proteins that have PDZ domains (Fig. 7.5). Assembly facilitates regulation of the transporters in the complex. For example, EBP50 (NHERF-1) and E3KARP (NHERF-2) participate in the PKA-dependent phosphorylation of CFTR and NHE3 [61] and NBC3 [57]. Notably, the interaction and mutual regulation of CFTR with the HCO_3^--secreting SLC26 transporters is mediated by the phosphorylated CFTR R domain and the SLC26 transporter STAS domain and is enhanced by the interaction of CFTR and SLC26 transporters with PDZ scaffolding proteins [78].

The findings above suggest that CFTR regulates fluid and HCO_3^- secretion both at rest and during stimulation. At rest, the HCO_3^- secretory mechanisms are not active while HCO_3^- salvage mechanisms absorb HCO_3^- at the luminal membrane. Upon cell stimulation, CFTR is activated and at the same time it inhibits HCO_3^- salvage and activates HCO_3^- secretory mechanisms at the luminal membrane to promote fluid and HCO_3^- secretion.

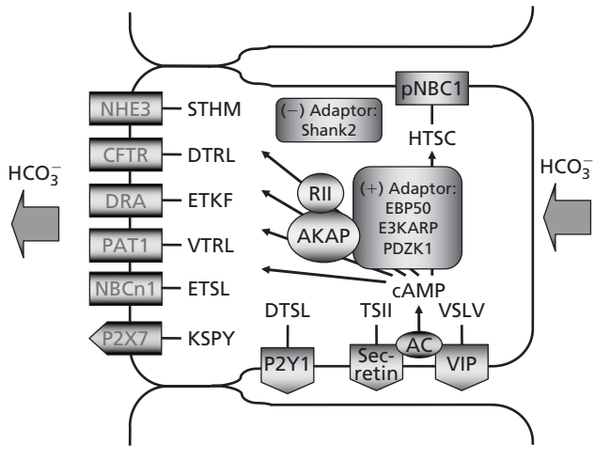

Figure 7.5 Assembly of HCO_3^- secretory complexes by PDZ-based adaptor proteins. Several membrane transporters and receptors have a unique carboxy-terminal sequence that forms a class I PDZ-binding motif (–T/S–X– hydrophobic amino acid), and bind to the PDZ domain-containing adaptor proteins to form HCO_3^--secreting complexes. Many of these proteins regulate, and are regulated by, CFTR to finely tune the secretory process. Secretion and the different modes of regulation are facilitated by formation of the complexes.

Other membrane transporters, pumps, and channels

The Na^+/K^+-ATPase pump is abundantly expressed in the basolateral membrane of the pancreatic duct and generates the primary driving force for transepithelial HCO_3^- secretion [79]. In addition, the Na^+/K^+-ATPase pump in conjunction with basolateral K^+ channels generate a negative membrane potential, which is essential for the electrogenic exit of HCO_3^- across the luminal membrane. Although several K^+ channels are active in pancreatic duct cells, the molecular identity of the major K^+ channel is not fully established. Maxi-K^+ channels at the basolateral membrane are the likely candidates for maintaining a negative membrane potential during HCO_3^- secretion [80]. Ca^{2+}-activated chloride channels are present in the luminal membrane of duct cells, although their role in HCO_3^- secretion is unclear.

In the past it was assumed that water followed the osmotic gradient and flowed from the basolateral to the luminal side via the paracellular pathway. However, it is now clear that water transport is mediated by the water channel aquaporins (AQP) and is a regulated process. Immunolocalization indicates expression of AQP1 at both the basolateral and the luminal membranes and AQP5 at the luminal membrane of pancreatic duct cells [81].

Regulation of membrane transporters

Fluid and electrolyte secretion by the pancreatic duct is a highly regulated process. The main stimulator of ductal secretion is secretin, which acts via an increase in cAMP and activation of PKA. The muscarinic receptors (M1 and M3), which act through changes in $[Ca^{2+}]_i$, augment the effect of secretin. The cAMP–PKA pathway activates or inhibits several basolateral membrane and luminal membrane HCO_3^- transporters. For example, phosphorylation of CFTR by PKA activates its anion channel function. In addition, the activation of luminal Cl^-/HCO_3^- exchange, basolateral pNBC, and maxi-K^+ channels by cAMP is well established [1]. On the other hand, the HCO_3^--absorbing transporters NHE3 and NBC3 are inhibited by activation of PKA [21]. The primary target of $[Ca^{2+}]_i$ in the duct is less certain, although a rise in $[Ca^{2+}]_i$ activates luminal Ca^{2+}-activated Cl^- channels and Cl^-/HCO_3^- exchange [39,40].

In recent years, a more complicated picture of the regulation of ductal fluid and electrolyte secretion has emerged with the realization that pancreatic cells express a multitude of receptors. These include multiple P2Rs [38] and the protease-activated receptor 2 (PAR2) [40,82], which may mediate critical steps in apoptosis (P2X7Rs) and the inflammatory response (PAR2) associated with pancreatitis. Functional studies suggest expression of P2Y2 and P2X7Rs at the luminal membrane and perhaps of P2Y1, P2Y2, and P2X4Rs at the basolateral membrane of the duct [38]. Both the P2Y and the P2X receptors signal through changes in $[Ca^{2+}]_i$ [31]. Subsequently, it has been reported that the luminal membrane P2X7Rs affect ductal pH_i and that the luminal membrane P2Rs stimulate, whereas the basolateral membrane P2Rs inhibit, HCO_3^- and fluid secretion in the guinea-pig duct [41]. P2Y11Rs that signal via changes in cAMP stimulate a luminal Cl^- channel, most likely CFTR. The basolateral PAR2 that signals through changes in $[Ca^{2+}]_i$ activates Ca^{2+}-activated Cl^- and K^+ channels [82] and stimulates HCO_3^- secretion [83] by the pancreatic duct.

Pancreatic ductal secretion is also subject to inhibitory inputs. The best documented is inhibition of ductal fluid and HCO_3^- secretion by substance P (SP). SP inhibits secretion by activation of PKC to inhibit luminal Cl^-/HCO_3^- exchange [84], which suggests that PKC should inhibit one or all the pancreatic SLC26 transporters. Indeed, PKC and PKC-activating agonists inhibit SLC26A6 activity by modulating its interaction with carbonic anhydrase (CA)II [85]. The effect of SP on other ductal SLC26 transporters is unknown.

Another class of proteins essential for pancreatic HCO_3^- secretion is the carbonic anhydrases. The CA inhibitor acetazolamide significantly inhibits secretion in humans and other species [86]. Originally, this finding was considered proof that CA generates most of the HCO_3^- during stimulated HCO_3^- secretion. However, it is now known that CA is present at the HCO_3^--transporting complex. It physically interacts with, and supplies HCO_3^- to regulate the activity of, several transporters such as NBC, AE, and SLC26A6 [87]. Immunolocalization revealed the presence of CAII, CAIV, CAIX, and CAXII in pancreatic duct cells. It is not clear which of these CAs is directly coupled to NBC and AE in the pancreatic duct. However, the trafficking of CAIV to the luminal membrane is dependent on CFTR, and acetazolamide inhibits HCO_3^- transport by SLC26A3 [88] and SLC26A6 [87]. These results imply involvement of ductal CAs in HCO_3^- transport by the CFTR–SLC26 transporter complexes.

Models and hypotheses of HCO_3^- secretion

Putting all the available information together has resulted in two different models of pancreatic duct fluid and HCO_3^- secretion. One was proposed by Steward et al. [1] and is reproduced in Fig. 7.6a, and the other was proposed by Ko et al. [60] and is summarized in Fig. 7.6b. The basic assumption of the model in Fig. 7.6a is that HCO_3^- secretion up to 70 mmol/L is mediated by electroneutral Cl^-/HCO_3^- exchange. Higher HCO_3^- concentration in the pancreatic juice is achieved by the action of CFTR as an HCO_3^- channel at the luminal membrane. The main tenet of the model in Fig. 7.6b is that two electrogenic Cl^-/HCO_3^- exchangers with isoform-specific stoichiometry mediate HCO_3^- secretion at different sites along the ductal tree. For example, a $1Cl^-/2HCO_3^-$ exchanger (e.g., SLC26A6) secretes the bulk of HCO_3^- and fluids in the proximal portion of the duct, while a $2Cl^-/1HCO_3^-$ exchanger (e.g., SLC26A3) concentrates HCO_3^- in the distal portion of the duct.

Significant differences between the models include the following.

1 The model in Fig. 7.6a requires inhibition of luminal and basolateral Cl^-/HCO_3^- exchangers, whereas the model in Fig. 7.6b requires electrogenic Cl^-/HCO_3^- exchange to concentrate HCO_3^- in the distal portion of the duct.

2 The model in Fig. 7.6a assumes that CFTR mediates HCO_3^- efflux in the distal duct, whereas the model in Fig. 7.6b proposes that CFTR determines the final HCO_3^- concentration in the pancreatic juice by preventing overconcentration of HCO_3^- by an electrogenic SLC26 transporter(s).

3 The model in Fig. 7.6a does not require Cl^-/HCO_3^- exchange for the bulk of ductal HCO_3^- secretion, whereas in the model shown in Fig. 7.6b ductal HCO_3^- secretion is dependent on luminal Cl^-/HCO_3^- exchange and CFTR has to be stimulated with SLC26 transporters for HCO_3^- secretion to take place.

Future studies analyzing the properties and role of all pancreatic SLC26 transporters should lead to the development of a comprehensive model of ductal HCO_3^- and fluid secretion.

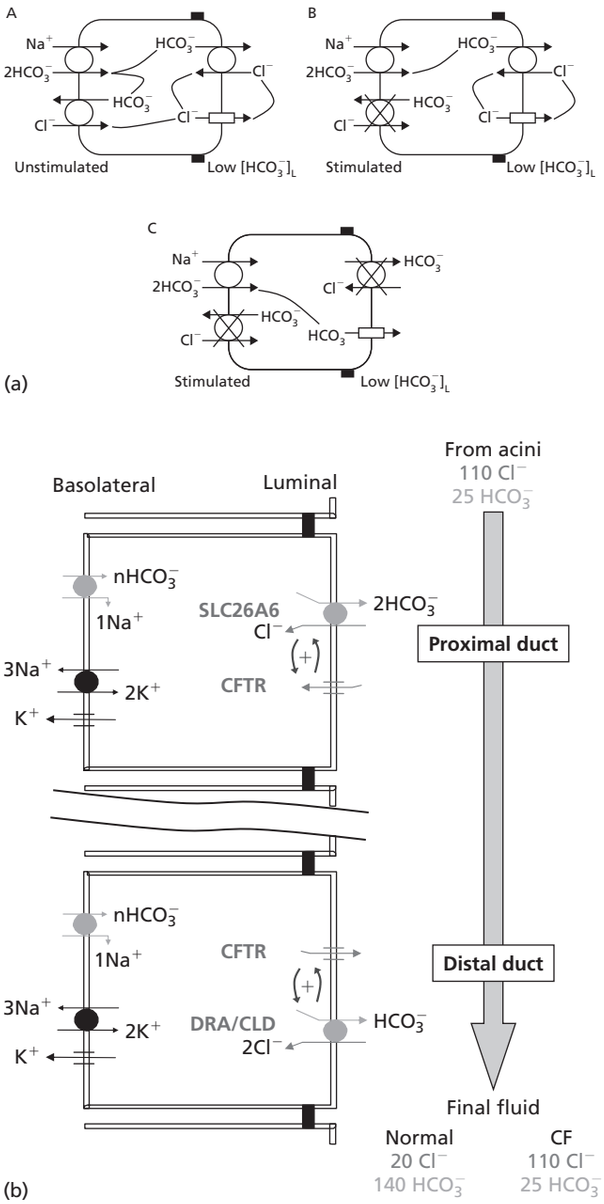

Figure 7.6 Models for fluid and HCO_3^- secretion by the pancreatic duct. (a) Model proposed by Steward et al. A, spontaneous secretion by an unstimulated duct cell; B, early stage of secretin-evoked secretion where the luminal Cl^-/HCO_3^- exchange mediates luminal HCO_3^- efflux; C, later stage of stimulated secretion, where a luminal HCO_3^- channel mediates HCO_3^- efflux. (From Ref. 1 with permission.) (b) Model proposed by Ko et al. Two electrogenic Cl^-/HCO_3^- exchangers with isoform-specific stoichiometry mediate HCO_3^- secretion at different sites along the ductal tree. (From Ref. 60 with permission.)

Clinical considerations

Cystic fibrosis and chronic pancreatitis

Cystic fibrosis is the most common autosomal recessive lethal single gene disorder in the Caucasian population. It is characterized by obstructive pulmonary and intestinal disorders, pancreatic insufficiency, fertility disorder, and high sweat Cl^-. Based on the mutation in CFTR, the disease phenotype varies in severity from mild to severe. The most common disease-causing mutation is deletion of phenylalanine at position 508 (ΔF508), which causes misfolding and degradation of CFTR, leading to its degradation by the ubiquitin–proteasome system. Although mutations in CFTR result in the reduction or absence of anion transport, the mechanism by which this leads to the pathogenesis of cystic fibrosis is less clear. Obstructions of the duct in the mucus-secreting glands of the lung, pancreatic duct, and epididymis by hyperviscous mucus result in their eventual destruction. The pancreatic juice of patients with cystic fibrosis contains a high concentration of Cl^- and is acidic [89]. Notably, several CFTR mutations associated with pancreatic

insufficiency retain substantial Cl^- channel activity, but lose their ability to support HCO_3^- secretion [59]. HCO_3^- is the biological pH buffer and as a chaotropic ion facilitates solubilization of mucins in the secreted fluid. Hence, aberrant ductal HCO_3^- secretion is likely a primary defect of cystic fibrosis.

Aberrant HCO_3^- secretion is also linked to chronic pancreatitis. A significant proportion of Caucasian and East Asian patients with chronic pancreatitis of unknown etiology possess a defective *CFTR* haploid gene [90,91]. Therefore, a mild defect in CFTR-dependent HCO_3^- transport appears to be an important predisposing factor in the development and/or progression of chronic pancreatitis.

Acute pancreatitis

A defining feature of acute pancreatic inflammation is the release of digestive enzymes into the pancreatic interstitium and the systemic circulation. Elevation of serum amylase is one of the indices of acute pancreatitis. Proteolytic enzymes, particularly trypsin, are autoactivated in acute pancreatitis. Activated trypsin can activate PAR2 in pancreatic, immune, and circulatory cells. Recent findings point to multiple and complicated effects of PAR2 in pancreatitis, in which intrapancreatic PAR2 protects the acinar and duct cells against pancreatitis-induced damage by activating survival signals, while mediating several systemic complications associated with pancreatitis through activation of PAR2 receptors in endothelial cells and macrophages [92]. Activation of PAR2 stimulates HCO_3^- transporters in the luminal membrane of pancreatic duct cells [40,82,83]. It is possible that PAR2-stimulated fluid secretion removes luminal toxic and inflammatory mediators during the early stages of acute pancreatitis.

Alcohol-associated diseases

A low concentration of ethanol markedly increases secretin-stimulated pancreatic fluid and HCO_3^- output in the guinea-pig duct, presumably by increasing cAMP and $[Ca^{2+}]_i$ [94]. However, a high concentration of alcohol has variable effects, depending on the protocol used and species. A recent finding of note in patients who had an attack of acute alcoholic pancreatitis [95] is a significant and persistent decrease in pancreatic juice HCO_3^- and enzyme content even 4–18 months after the attack. This raises the possibility that chronic compromised pancreatic function, including HCO_3^- secretion, may be associated with a propensity to acute alcoholic pancreatitis. In support of this possibility is the finding that *CFTR* gene polymorphism is associated with chronic alcoholic pancreatitis [6,96]. Further work is needed to determine a possible link between the status of pancreatic function and the pathogenesis of alcohol-associated pancreatitis.

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