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Molecular Mechanism of Pancreatic Bicarbonate Secretion

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Thanks to recent progress in availability of molecular and functional techniques it became possible to search for the basic molecular and cellular processes that mediate and control HCO_3^- and fluid secretion by the pancreatic duct. The coordinated action of various transporters on the luminal and basolateral membranes of polarized epithelial cells mediates the transepithelial HCO_3^- transport, which involves HCO_3^- absorption in the resting state and HCO_3^- secretion in the stimulated state. The overall process of HCO_3^- secretion can be divided into two steps. First, HCO_3^- in the blood enters the ductal epithelial cells across the basolateral membrane either by simple diffusion in the forms of CO_2 and H_2O or by the action of an Na^+-coupled transporter, a Na^+-HCO_3^- cotranporter (NBC) identified as pNBC1. Subsequently, the cells secrete HCO_3^- to the luminal space using at least two HCO_3^- exit mechanisms at the luminal membrane. One of the critical transporters needed for all forms of HCO_3^- secretion across the luminal membrane is the cystic fibrosis transmembrane conductance regulator (CFTR). In the resting state the pancreatic duct, and probably other HCO_3^- secretory epithelia, absorb HCO_3^-. Interestingly, CFTR also control this mechanism. In this review, we discuss recent progress in understanding epithelial HCO_3^- transport, in particular the nature of the luminal transporters and their regulation by CFTR.

Key Words: Pancreas, Bicarbonate, CFTR, Transporter

INTRODUCTION

Pancreatic duct secretes bicarbonate-rich fluid, which is important in maintaining the patency of ductal tree, as well as intestinal digestive function. Pancreatic HCO_3^- protects the duodenal epithelia by neutralizing gastric acid and aids in maintaining an optimal pH for the function of digestive enzymes (Argent & Case, 1994). Accumulating evidence suggests that secretion of the HCO_3^--rich fluid is also important in protecting the intrapancreatic ductal tree. Transepithelial Cl^- and HCO_3^- transport are the principal driving force for fluid secretion by ductal cells and reduced HCO_3^- concentration results in acidification of the luminal environment. The rheologic properties of mucins are affected by mucin concentration and the pH of the solvent. In general, mucin precipitation and viscosity progressively increase as the pH and volume of the secreted fluid decrease. Indeed, the most prominent change in the composition of the pancreatic juice of obstructive ductal diseases, such as cystic fibrosis (CF) or chronic pancreatitis, is a reduction in HCO_3^- concentration of the secreted fluid (Choi et al, 2001).

Recent technical advances, such as the use of microperfused ducts, molecular identification of transporters and the availability of genetically modified mice, permitted close examination of the basic cellular processes responsible for the formation and secretion of a HCO_3^-- rich fluid by epithelial cells such as those of the pancreatic duct. In this review, after a brief introduction of current concepts on the basic molecular mechanisms of pancreatic bicarbonate secretion, we will focus on recent progress made in understanding the luminal HCO_3^- transport mechanisms, with an emphasis on the role of cystic fibrosis transmembrane conductance regulator (CFTR) in this processes and their pathophysiological implications.

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The overall HCO_3^- secretory process

The coordinated action of various transporters in the luminal and basolateral membranes of polarized epithelial cells mediates transepithelial HCO_3^- transport. The overall HCO_3^- secretory process can be divided into two steps (Fig. 1). The first step encompasses the entry of blood c into pancreatic duct cells across the basolateral membrane. Early studies suggested that most of the bicarbonate is generated in the duct by carbonic anhydrase (CA). As depicted in Fig. 1, CA hydrates the diffused CO_2 to form the volatile carbonic acid, which dissociates to H^+ and HCO_3^-. To secrete HCO_3^- to the lumen, H^+ generated in the cytosol must be disposed off by transport back to the blood. There is good molecular and functional evidence that

ABBREVIATIONS: CFTR, cystic fibrosis transmembrane conductance regulator; AE, Cl^-/HCO_3^- exchanger; NHE, Na^+/H^+ exchanger; NBC, Na^+, HCO_3 cotransporter; CA, carbonic anhydrase.

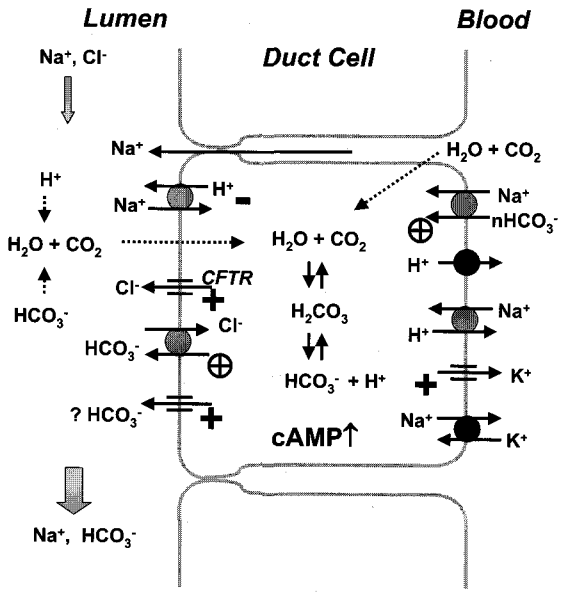

Fig. 1. A model for HCO_3^- secretion in pancreatic duct cells. +: activation by cAMP, ⊕: indirect activation through CFTR, −: inhibition by cAMP.

an electrogenic H^+-ATPase pump and a Na^+/H^+ exchanger (NHE) are expressed in basolateral membrane. However, their role in bicarbonate secretion is fully agreed upon. Several studies reported that inhibitors of the H^+-ATPase and NHE reduced pancreatic HCO_3^- secretion, while others reported no effects of these compounds. Indeed, a recent computer modeling suggested that H^+-ATPase and basolateral NHE are responsible for only 4% and 15% of H^+-back leak (or HCO_3^- influx) during agonist-stimulated secretion, respectively (Sohma et al, 2000).

More direct results obtained by microperfusion of the rat pancreatic duct (Zhao et al, 1994), and later confirmed in the guinea pig pancreatic duct as well (Ishiguro et al, 1998), indicate that most of HCO_3^- transport across basolateral membrane during active HCO_3^- secretion is achieved by a Na^+-coupled HCO_3^- transporter, which mediates a Na^+-HCO_3^- cotransport (NBC). Basolateral application of the NBC inhibitor, H_2DIDS inhibits 56% of HCO_3^- secretion, while inhibitor of NHE inhibits only 18% of the secretion. Subsequently the pancreatic isoform of the electrogenic family of NBCs, pNBC, was cloned and localized in the basolateral membrane of pancreatic (Abuladze et al, 1998) and salivary gland acinar and duct cells (Luo et al, 2001). In the computer model, pNBC can mediate as much as 80% of HCO_3^- influx during active HCO_3^- secretion (Sohma et al, 2000). The stoichiometry of pNBC was estimated as 1 Na^+ : 2 HCO_3^-. Since it is electrogenic, depolarization induced by cAMP-dependent activation of CFTR (see below) facilitates HCO_3^- influx through pNBC.

In the luminal membrane, CFTR and Cl^-/HCO_3^- exchange activity (AE: anion exchange) are likely the main transporters mediating the luminal exit of HCO_3^-. Increase in intracellular cAMP by secretin and VIP (vasoactive intestinal polypeptide), is the major signal for stimulation of pancreatic HCO_3^- secretion. Activation of CFTR Cl^- channel by the increase in cellular cAMP and a concurrent AE activity are responsible for the cAMP-induced HCO_3^- secretion. Under optimal conditions and HCO_3^--dependent inhibition of AE activity, this model can explain fluid secretion containing up to 70~120 mM. However, in some species, such as human and guinea pig, pancreatic HCO_3^- concentration reaches 140 mM. Therefore, it is conceivable that other electrogenic luminal HCO_3^- exit mechanism, such as HCO_3^- channel, are expressed and participate in HCO_3^- efflux across the luminal membrane. Indeed, it was shown that cAMP increases an electrogenic HCO_3^- permeability in the luminal membrane of guinea pig pancreas, albeit the molecular identity of which is unknown (Ishiguro et al, 1998).

Also unknown with certainty are the nature of the luminal AE. However, recently, we reported that the activity of luminal AE is dependent on the expression and activation of CFTR (Lee et al, 1999a; 1999b). Considering the fact that disruption of CFTR causes obstructive ductal diseases such as cystic fibrosis (CF) or chronic pancreatitis, this finding may have implications to the pathophysiological mechanism for such diseases. The original observation of luminal NHE activity in the luminal membrane of the duct (Zhao et al, 1994) appeared problematic. A priory, this activity appeared to be counter-productive to the main function of the duct. However, subsequent work confirmed these observations and extended them to show that in addition to HCO_3^- secretory mechanisms, the pancreatic duct and other HCO_3^- secretory cells express HCO_3^- absorbing mechanisms such as NHE3 (Lee et al, 2000) and the electroneutral NBCs (Luo et al, 2001). Interestingly, the HCO_3^- secreting AE activity and the HCO_3^- absorbing NHE3 are closely associated with CFTR to coordinate the entire HCO_3^- transport in the luminal membrane (Ahn et al, 2001). Below, we will discuss these findings and their potential pathophysiological consequences.

Activation of Cl^-/HCO_3^- exchange by CFTR

Pancreatic HCO_3^- secretion is impaired in patients with cystic fibrosis or chronic pancreatitis due to mutations in the CFTR gene. Since the major mechanism of HCO_3^- secretion in CFTR-expressing cells is mediated by the action of a Cl^-/HCO_3^- exchanger (AE), the possible regulation of AE activity by CFTR was examined.

In the first set of experiments, we studied regulation of AE activity by CFTR in a heterologous expression system (Lee et al, 1999a). We used stably transfected NIH 3T3 cells that express high levels of the CFTR protein. Mock-transfected cells of the same parental line were used as controls. A standard protocol of removal and addition of Cl^- to the incubation medium buffered with HCO_3^- was used to follow Cl^-/HCO_3^- exchange activity. Fig. 2 illustrates the basic observation in which CFTR expressing cells exhibited a forskolin stimulated AE activity. Removal of Cl^- from the incubation medium of mock-transfected cells resulted in a slow and modest increase in pH_i, which was completely reversed on addition of Cl^- to the medium. Stimulation of control cells with 5 μM forskolin had no effect on basal level of pH_i or the pH_i changes observed upon removal and re-addition of Cl^-. Stimulation of CFTR expressing 3T3 cells with forskolin caused a time-dependent intracellular acidification that was complete after 3 min of incubation at 37°C. The nature of this acidification is not known. However, it was clearly dependent on the expression of CFTR and was markedly reduced or abolished by depo-

larizing the cells with high bath K^+, suggesting that it might reflect conductive HCO_3^- transport by CFTR. Most notably, removal of Cl^- from the incubation medium of forskolin-stimulated, CFTR expressing cells caused a rapid and a large increase in pH_i that was reversed upon re-addition of Cl^- to the medium. After forskolin stimulation, the rate of pH_i change due to the changes in transcellular Cl^- gradient in CFTR expressing cells was 8 fold faster than that of the same cells before forskolin stimulation, or before and after forskolin stimulation in control cells ($P<0.01$). Thus, the increased rate of pH_i changes required both the expression of CFTR and the activation of the protein by a cAMP-dependent mechanism. It is well established that CFTR mediated Cl^- channel activity is regulated by a cAMP-dependent phosphorylation. However, the increased pH_i in CFTR-expressing cells shown in Fig. 2 appears to be mediated largely by activation of an electroneutral AE, rather than an electrogenic mechanism.

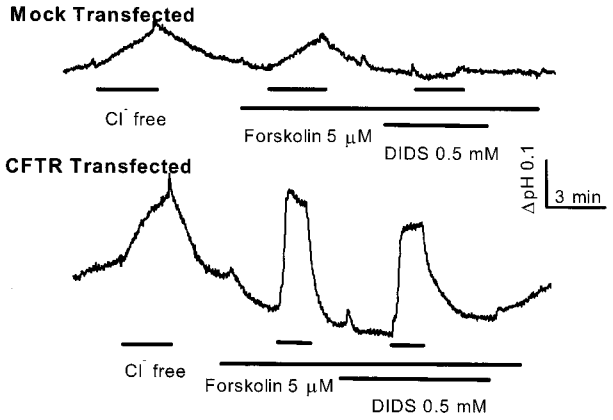

Fig. 2. CFTR activates Cl^-/HCO_3^- exchange activity in NIH 3T3 cells stably expressing CFTR. Cl^-/HCO_3^- exchange activity was estimated from the rate of changes in pH_i. Note that stimulation of CFTR with forskolin was needed to activate AE activity (adopted with permission from Lee et al, 1999a).

The findings in expression systems were extended to cell lines and native tissues expressing CFTR (Lee et al, 1999b). The cell line of choice was the intestinal T84 since CFTR activity in this line is well characterized. As in the heterologous expression system, stimulation of T84 cells with forskolin increased the activity of the Cl^-/HCO_3^- exchanger independent of the Cl^- channel activity of CFTR. More important, freshly isolated submandibular gland ducts from wild type mice showed separate luminal and basolateral AE activity. Membrane depolarization with 5 mM Ba^{2+} or 100 mM K^+ was used to increased and stabilized $[Cl^-]_i$ and isolate the electroneutral component of the AE activity in both membranes. Under depolarized conditions wild type and $\Delta F/\Delta F$ mice had comparable basal AE activities. Notably, stimulation with forskolin increased AE activity in submandibular gland ducts from wild type but not in those from CFTR-deficient $\Delta F/\Delta F$ mice. Microperfusion of the main pancreatic duct showed AE activities in both the basolateral and luminal membranes. Stimulation of ducts from wild type animals with forskolin had no effect on basolateral but markedly stimulated luminal AE activity. By contrast, forskolin had no effect on either basolateral or luminal Cl^-/HCO_3^- exchange activity in ducts from $\Delta F/\Delta F$ animals (Fig. 3). These and similar findi-

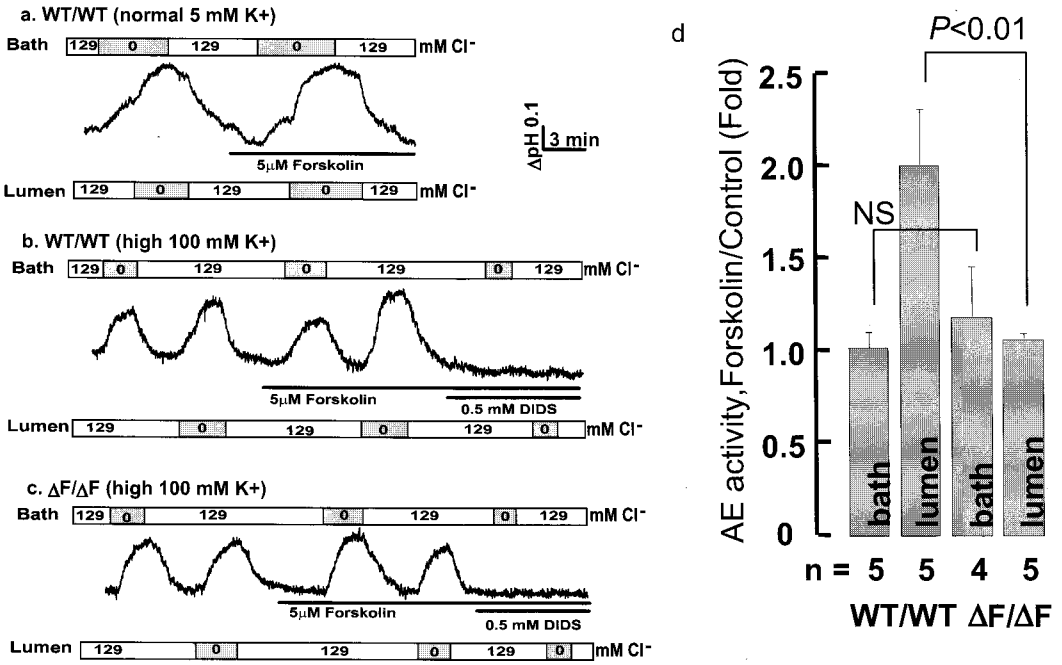

Fig. 3. CFTR stimulates the luminal but not the basolateral AE activity in the perfused pancreatic ducts. The main pancreatic ducts of WT (*a, b*) and $\Delta F/\Delta F$ (*c*) mice were used to evaluate Cl^-/HCO_3^- exchange activity. Note that stimulation of CFTR activated luminal Cl^-/HCO_3^- exchange activity only in ducts from WT mice (adopted with permission from Lee et al, 1999b).

ngs led to the conclusion that CFTR activates Cl^-/HCO_3^- exchange activity in HCO_3^- secretory epithelia.

The importance of the CFTR-stimulated, Cl^--dependent HCO_3^- transport to the function of secretory epithelia and CF was revealed by comparing effects of mutations in CFTR associated with CF on Cl^- channel activity and HCO_3^- transport. CFTR is a cAMP-regulated Cl^- channel and most CF-causing mutations in CFTR inhibit Cl^- channel activity. However, identification of several CF-causing mutants with substantial or normal Cl^- channel activity indicates that other CFTR-dependent processes contribute to manifestation of the disease. Therefore, it was of interest to examine Cl^--coupled HCO_3^- transport by CF-associated CFTR mutants that retain substantial or normal Cl^- channel activity. In general, all mutations in CFTR inhibited Cl^--dependent HCO_3^- transport more than Cl^- channel activity, supporting the notion that CFTR mediate two separate activities. Furthermore, although the correlation was not perfect, the trend found was that mutations in CFTR reported to cause CF with severe clinical presentations did not support HCO_3^- transport and those with mild clinical presentations showed reduced HCO_3^- transport (Choi et al, 2001). Fig. 4 shows the correlation between the reported pancreatic status of the patients and the HCO_3^-/Cl^- transport ratio. When the ratio measured with CFTR is taken as 1, mutants that cause a severe form of CF show an HCO_3^-/Cl^- transport ratio of less than 0.1. By comparison, most mutations that cause a mild form of CF showed an HCO_3^-/Cl^- transport ratio between 0.31~0.46. Consequently, the CFTR-dependent HCO_3^-/Cl^- transport ratio appears to correlate reasonably well with the reported pancreatic status of CF patients. It is important to note that altered HCO_3^- transport show particular correlation with pancreatic function since the pancreas secret copious amount of HCO_3^- (Argent & Case, 1994). The same may not hold for other tissues, for example the sweat gland, since fluid secreted by the sweat gland contains less HCO_3^- than the systemic HCO_3^- concentration. The aberrant HCO_3^- transport by the CF-causing mutations examined indicates that HCO_3^- transport by CFTR-expressing epithelia is critical for normal tissue physiology and that impaired HCO_3^- transport is sufficient to derange the pancreatic function even in the presence of Cl^- channel activity.

A search for the mechanism in the luminal membrane that mediate the AE activity revealed that secretory epithelia do not express members of the classical AE family SLC4. On the other hand colonocites (Silberg et al, 1995), the renal proximal tubule (Knauf et al, 2001) and the auditory epithelia (Everett et al, 1997) and β-intercalated cells (Royaux et al, 2001) express members of the newly discovered family of Cl^-/HCO_3^- exchangers of SLC26. Based on this finding it was logical to test the effect of CFTR on the activity of these Cl^-/HCO_3^- exchanges. Preliminary work showed that CFTR markedly activate Cl^-/HCO_3^- exchange by all members of the SLC26 tested (Ko et al, 2002). Confirmation of these findings and revealing the mechanism by which CFTR activates the SLC26 exchangers will undoubtedly clarify the mechanism by which CFTR regulate the electroneutral portion of HCO_3^- secretion of secretory epithelia.

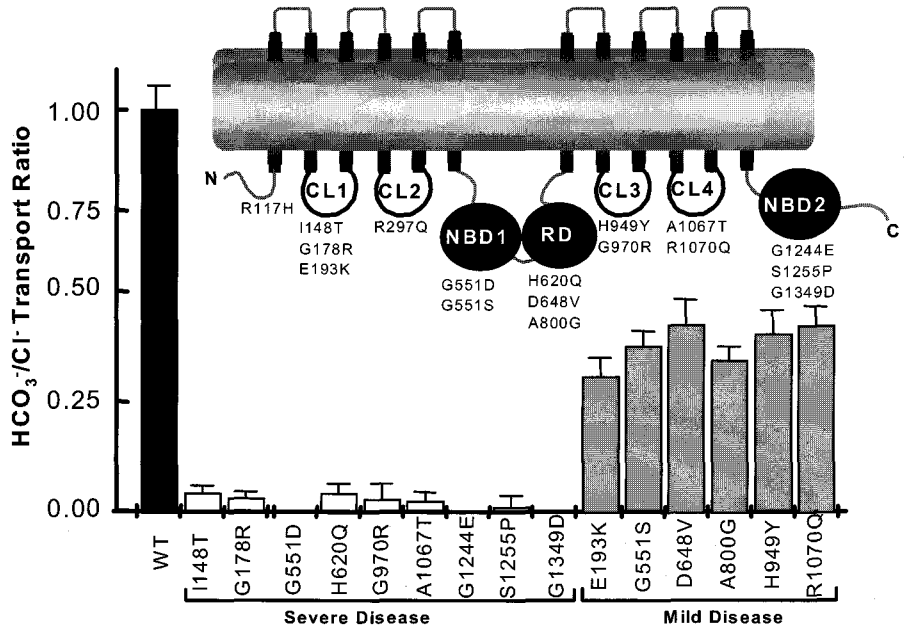

Fig. 4. The HCO_3^-/Cl^- transport ratio of CFTR mutants associated with CF. The HCO_3^-/Cl^- transport ratios were calculated from the rates of net Cl^- and HCO_3^- transport. The ratio measured in WT CFTR was set to 1. The inset schematically illustrates the different cytoplasmic domains of CFTR. CL1, Cytoplasmic Loop 1; CL2, Cytoplasmic Loop 2; NBD1, Nucleotide Binding Domain 1; RD, Regulatory Domain; CL3, Cytoplasmic Loop 3; CL4, Cytoplasmic Loop 4; NBD2, Nucleotide Binding Domain 2 (adopted from Choi et al, 2001 with permission from Nature publishing group).

Regulatory HCO_3^- absorption by luminal mechanisms

An interesting feature of HCO_3^- transport in HCO_3^--secreting epithelia is the existence of regulatory HCO_3^- absorptive pathways in the luminal membrane. Prominent among them is a Na^+/H^+ exchange activity. Na^+/H^+ exchange activity is mediated by members of the NHE family of Na^+/H^+ exchangers. To date 7 Na^+/H^+ exchangers were identified, which are mostly expressed in the plasma membrane, with several isoforms residing in intracellular organelles (Miyazaki et al, 2001). The best characterized NHEs are NHE1, NHE2 and NHE3. The house keeping NHE1 is expressed virtually in all cells and is always in the basolateral membrane of epithelial cells. NHE2 and NHE3 are expressed in the luminal membrane of epithelial cells. In spite of its wide spread expression in the luminal membrane of epithelial cells, the role of NHE2 remain a mystery (Lee et al, 1998). Deletion of NHE2 had minimal a physiological phenotype (Schultheis et al. 1998). NHE3 is the exchanger mediating about 50% of Na^+ and HCO_3^- absorption in the renal proximal tubule (Choi et al, 2000).

Originally, a paradoxical NHE activity was found in the luminal membrane of the pancreatic duct (Zhao et al, 1994). Subsequently this activity was identified as mediated by NHE3 and a novel Na^+-dependent $H^+/OH^-/HCO_3^-$ transporter in the pancreatic (Lee et al, 2000) and submandibular ducts (Luo et al, 2001). The H^+ efflux/HCO_3^- influx mechanisms were characterized in pancreatic ducts from wild type (WT), $NHE2^{-/-}$ and $NHE3^{-/-}$ mice. RT-PCR analysis in combination with immunolocalization showed that the pancreatic duct expresses NHE1 in the basolateral membrane and NHE2 and NHE3 in the luminal membrane. Measurement of Na^+-dependent H^+ efflux in the microperfused duct demonstrated a basolateral activity inhibited by 1.5 μM of the AE1 inhibitor HOE 694, consistent with expression of NHE1, and a luminal activity inhibited by 50 μM HOE 694, consistent with expression of NHE2. However, disruption of the *NHE2* gene had no effect on luminal transport. By contrast, disruption of the *NHE3* gene reduced luminal Na^+-dependent H^+ efflux by about 45%. Notably, the remaining luminal Na^+-dependent H^+ efflux was not inhibited in ducts from $NHE2^{-/-}$; $NHE3^{-/-}$ double knockout mice (Choi et al, 2000; Lee et al, 2000). Therefore, nearly 55% of luminal H^+ efflux in the pancreatic duct is mediated by a novel, HOE 694 (or amiloride)-sensitive, Na^+-dependent mechanism. H^+ transport by the two luminal mechanisms is inhibited by cAMP stimulation, albeit to a different extent.

A potential mediator of the novel Na^+-dependent H^+ efflux mechanism is one or a combination of splice variants of the electroneutral NBCs, NBCn1A-NBCn1D (Choi et al, 2000). PR-RT-PCR analysis and immunolocalization showed that the submandibular gland (Luo et al, 2001) and pancreatic (MGL and SM, unpublished observations) ducts express at least one NBCn1 splice variant in the luminal membrane. Furthermore, experiments in microperfused ducts revealed an activity with properties resembling those of NBCn1 was found in the luminal membrane of the duct. However, it remained to be determined whether any NBCn1 splice variant can account for this activity and whether regulation of the activity by elevation of cAMP (Luo et al, 2001) is mediated by CFTR.

Another aspect of the interaction between CFTR and the $H^+/OH^-/HCO_3^-$ transporters is the possibility of reciprocal regulation of their activity. As will be discussed below, CFTR and the HCO_3^- transporters exist in the same HCO_3^- transporting complex. The complex is assembled with the aid of scaffolding proteins. The scaffolding proteins are not inert. They actually affect the activity of CFTR. Thus multimerization of CFTR by binding to the two PDZ binding domains of EBP50 (Raghuram et al, 2001) of PDZK (Wang et al, 2000) increase channel activity. This raises the possibility that interaction of CFTR and one of the HCO_3^-

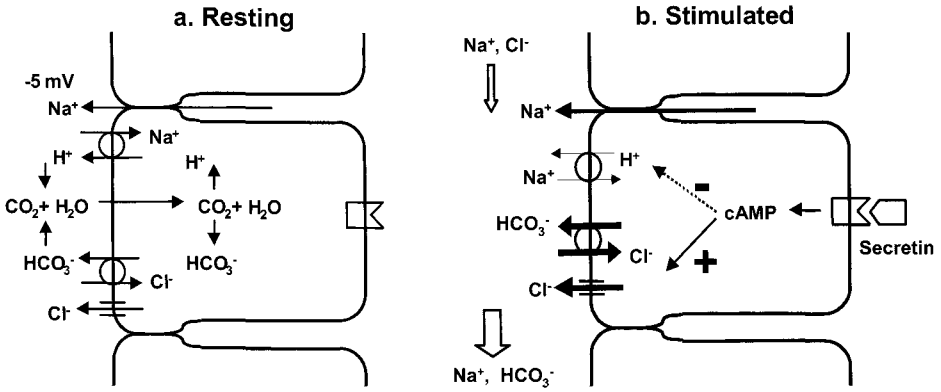

Fig. 5. The role of luminal Na^+-dependent H^+ efflux mechanisms in pancreatic ductal HCO_3^- transport. A) The resting pancreatic duct maintains a transepithelial voltage of -5 mV, lumen negative and has a leaky tight junction. Hence, Na^+ flows from the interstitial to the luminal space. The luminal Na^+-dependent H^+ efflux mechanisms absorb the Na^+ in exchange for H^+ (or in coupling with HCO_3^- influx). H^+ efflux is equivalent to HCO_3^- re-absorption. HCO_3^- can originate in luminal Cl^-/HCO_3^- exchange activity of the resting duct (slippage mode), or present in the fluid secreted by acinar cells. B) Upon feeding, the gastrointestinal hormone secretin is released from neuroendocrine intestinal cells and stimulates pancreatic secretion by increasing intracellular cAMP in duct cells. The luminal Na^+-dependent H^+ efflux mechanisms are down regulated to reduce Na^+-HCO_3^- re-absorption (adopted from Lee et al, 2000 with permission).

transporters will disrupt/minimize multimerization of CFTR to reduce channel activity. This possibility was examined recently. As expected, the expression of rat NHE3 significantly decreased PKA-dependent activation of CFTR without altering CFTR expression and the decrease in activity was prevented by mutation of either of the two NHE3 PKA targets (Bagorda et al, 2002).

Based on the findings summarized above, it is possible to propose that multiple Na^+-dependent H^+ efflux mechanisms in the luminal membrane of the pancreatic and submandibular gland ducts and likely other epithelia absorb Na^+ and HCO_3^- to produce a HCO_3^--poor and Cl^--rich fluid during basal secretion. Inhibition of the transporters during active pancreatic or salivary secretion aids in producing the HCO_3^--rich and Cl^--poor fluid.

HCO_3^- transport complexes

As stated above, the pancreatic and submandibular gland ducts express CFTR-dependent HCO_3^- secretory and an NHE3-mediated HCO_3^- absorptive mechanism in the luminal membrane. Regulatory interaction between all transporters is possible, since CFTR and NHE3 interact with cellular scaffolding proteins such as EBP50 (NHERF1) and E3KARP (NHERF2). This possibility was tested directly by molecular, biochemical and functional approaches in heterologous expression systems and in the native pancreatic duct. When present in the same membrane, CFTR regulates NHE3 activity by both acute and chronic mechanisms. In the pancreatic duct CFTR affected expression of NHE3 in the luminal membrane. Thus, luminal expression of NHE3 was reduced by 53% in ducts of homozygote DF508 mice. Accordingly, luminal Na^+-dependent and HOE-sensitive recovery from an acid load was reduced by 60% in ducts of ΔF508 mice. CFTR and NHE3 were co-immunoprecipitated from PS120 cells expressing both proteins and the pancreatic duct of wild type mice, but not from PS120 cells lacking CFTR or the pancreas of DF508 mice. The interaction between CFTR and NHE3 required the COOH-terminal PDZ binding motif of CFTR. Mutant CFTR constructs lacking the C-terminus were not co-immunoprecipitated with NHE3. Furthermore, when expressed in PS120 cells, wild type CFTR, but not CFTR mutants lacking the C-terminal PDZ binding motif, augmented cAMP-dependent inhibition of NHE3 activity by 31% (Fig. 6).

These findings provide initial evidence for the existence of a HCO_3^- transport regulatory complex with CFTR in its center. By analogy with assembly of the PSD-95 (Kim et al, 1995), it is likely that such a complex is assembled with the aid of several scaffolding proteins. Indeed, the complex contains at least two scaffolding proteins, an EBP50 like and at least one AKAP. All known AKAPs have multiple protein-protein interacting domain and function as scaffolding proteins (Michel & Scott, 2002). In the complex a coordinated regulation of HCO_3^- secretion is mediated by interaction of CFTR with several HCO_3^- transporters as was found for the CFTR-NHE3 protein complex. In this respect, it is of particular interest that many of the G protein-coupled receptors and transporters related to HCO_3^- secretion in secretory epithelial cells have a PDZ-binding motif on their C-terminus (Fig. 7). In addition, most of them are associated with cAMP-dependent processes. As noted above, not only CFTR regulates the activity of the HCO_3^- transporters, but also binding of the transporters can regulate the activity of CFTR. Therefore, precise understanding of protein interactions between members of the HCO_3^- transporting complex in the future will contribute to elucidate the overall regulatory mechanism of epithelial HCO_3^- transport.

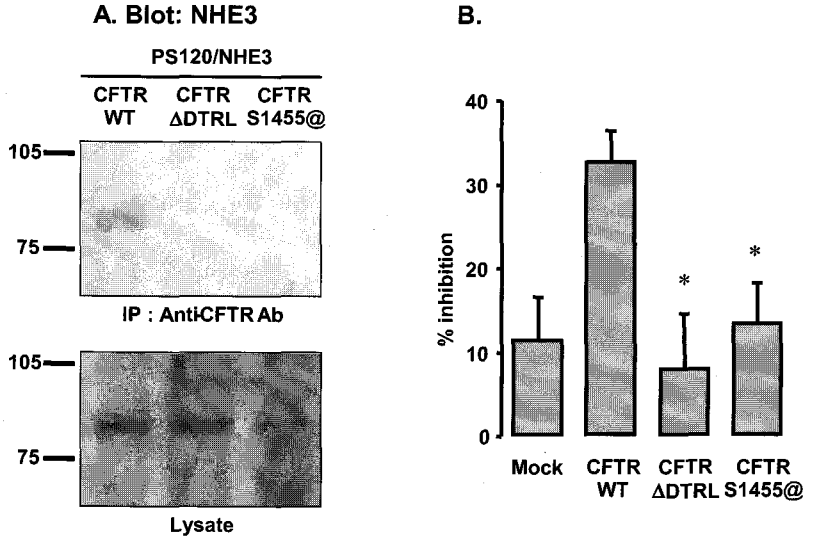

Fig. 6. The PDZ binding motif in the C-terminus of CFTR is required for formation of CFTR-NHE3 complexes and regulation of NHE3 activity by CFTR. WT and mutant CFTR were expressed in cells stably expressing NHE3 to demonstrate that cAMP-dependent stimulation of CFTR inhibits NHE3 activity and the role of the PDZ binding motif in this function of CFTR (adopted from Ahn et al, 2001 with permission).

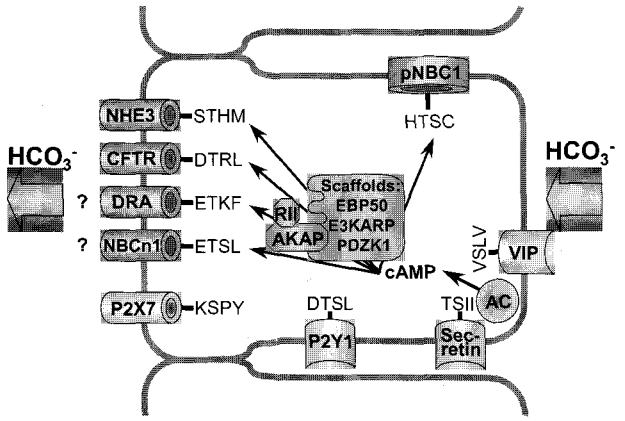

Fig. 7. A putative HCO_3^- transporting complex. Several G protein coupled receptors and transporters participating in HCO_3^- homeostasis in pancreatic duct cells have a PDZ-binding motif (-X-T/S-X-hydrophobic amino acid) at their C-terminus. The C-terminal sequences are based on the human clones (modified from Lee et al, 2001).

Concluding remarks

Acidic fluid secretion due to a defect in epithelial HCO_3^- transport could lead to precipitation of mucin and plugging of ductal system and facilitate bacterial infection *via* binding to the precipitated mucin. In the special case of the pancreas, acidic pH would lead to premature activation of digestive enzymes, destruction of the pancreas and pancreatic insufficiency. Thus, aberrant HCO_3^- transport can account for the diverse pathological states observed in the obstructive diseases of ductal system such as CF, bronchiectasis and chronic pancreatitis. Recent findings suggest that enhancing HCO_3^- transport by epithelial cells or increasing the HCO_3^- content on the apical surface of affected tissues might be considered as additional means of ameliorating the debilitating effects of these diseases.

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