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Coordination of Pancreatic HCO_3^- Secretion by Protein-Protein Interaction between Membrane Transporters

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Summary

Increasing evidence suggests that protein-protein interaction is essential in many biological processes including epithelial transport. In this report, we discuss the significance of protein interactions to HCO_3^- secretion in pancreatic duct cells. In pancreatic ducts HCO_3^- secretion is mediated by cystic fibrosis transmembrane conductance regulator (CFTR) activated luminal Cl^-/HCO_3^- exchange activity and HCO_3^- absorption is achieved by Na^+-dependent mechanisms including Na^+/H^+ exchanger 3 (NHE3). We found biochemical and functional association between CFTR and NHE3. In addition, protein binding through PDZ modules is needed for this regulatory interaction. CFTR affected NHE3 activities in two ways. Acutely, CFTR augmented the cAMP-dependent inhibition of NHE3. In a chronic mechanism, CFTR increases the luminal expression of Na^+/H^+ exchange in pancreatic duct cells. These findings reveal that protein complexes in the plasma membrane of pancreatic duct cells are highly organized for efficient HCO_3^- secretion.

Fluid secretion is required for proper functioning of essential organs such as the lung and pancreas. HCO_3^-, an important component of the secreted fluids, is the subject of increased attention since it governs the luminal pH and solubility of protein in the secreted fluids. We have previously reported that cystic fibrosis transmembrane conductance regulator (CFTR) participates in HCO_3^- secretion by stimulating a Cl^-- dependent HCO_3^- transport, in the form of Cl^-/HCO_3^- exchange activity [1, 2]. Another important mechanism in HCO_3^- homeostasis is a HCO_3^--absorbing processes in the resting state. In the pancreatic duct 50% of HCO_3^- absorption is mediated by Na^+/H^+ exchanger 3 (NHE3) and 50% by a novel, yet unidentified, Na^+-dependent mechanism [3]. An interesting feature of HCO_3^- homeostasis is the possibility that the activity of multiple mechanisms is regulated by interaction between the transporters mediated by scaffolding proteins such as ezrin-binding phosphoprotein 50 (EBP50) [4]. Both PDZ (PSD95, Dlg1, ZO-1) domains of EBP50 bind the C-terminus of CFTR to dimerize it and regulate its activity as a Cl^- channel [5]. NHE3 interacts with EBP50 via the second PDZ domain [6]. In a recent work, we observed regulatory interaction between CFTR and NHE3, possibly through EBP50, in a heterologous expression system of PS120 cells and in the native pancreatic duct [7]. Here, we discuss the significance of protein

interactions to HCO_3^- secretion in pancreatic duct cells.

Initially, we examined whether CFTR and NHE3 exist in the same protein complexes. NHE3 was found in the anti-CFTR immunoprecipitates when CFTR and NHE3 were co-expressed in PS120 cells, demonstrating that exogenously expressed CFTR and NHE3 may associate in a stable complex. To determine whether CFTR and NHE3 also associate in native cells, we performed the same experiments using pancreata from wild type (WT) and CFTR-impaired homozygote ΔF508 (ΔF) mice. NHE3 was detected in anti-CFTR immunoprecipitates from the pancreas of WT mouse. In contrast, only a very small amount of NHE3 was found in CFTR immunoprecipitates from the pancreas of ΔF mouse.

Next we studied the effect of CFTR on NHE3 activity. Treatment of PS120/NHE3 cells with forskolin inhibited NHE3 activity dose-dependently, which was maximal at 10 μM. Forskolin also inhibited NHE3 activity in CFTR co-expressing cells. However, the inhibition of NHE3 activity was significantly higher at any given forskolin concentrations when compared to control cells and nearly maximum at 0.1 μM of forskolin. Thus, we concluded that activation of CFTR augments cAMP-mediated inhibition of NHE3 in PS120 cells.

In an immunolocalization study, we observed the co-localization of CFTR, NHE3, and EBP50 in the luminal area of mouse pancreatic duct cells. Therefore we determined whether CFTR expression affects the Na^+/H^+ exchange activity in the luminal membrane of the perfused pancreatic duct. When the luminal NHE3 activity was measured in pancreatic ducts from ΔF mice, it was evident that the basal activity was significantly lower than that from WT mice. The reduced activity in ΔF mice was independent of age. Similar degree of reduction in NHE3 activity was found in as early as 2-week-old mice, suggesting that an innate mechanism is responsible for the decreased activity rather than an adaptive process necessary for survival (Table 1). Subsequent quantitative confocal microscopy revealed 53% reduced luminal expression of NHE3 in ducts from ΔF mice. In another set of experiment using mice ages from 3 to 6 months, we found that 10 μM forskolin inhibited the luminal NHE3 activity by 40% in WT mice, similar to the findings in PS120 cells. However, the same concentration of forskolin failed to show significant inhibition on the residual NHE3 activity in ΔF mice.

The present findings may have importance in understanding the overall role of CFTR in epithelial physiology and in cystic fibrosis. Notably, co-expression of CFTR increased the basal activity and expression levels of NHE3 in the luminal membrane of pancreatic duct cells. By forming a protein complex, CFTR may enhance the stability of the expressed NHE3 or its delivery to the luminal membrane of the pancreatic duct. Alternatively, CFTR may increase the transcription of NHE3 mRNA or its half-life. In an acute mechanism, CFTR augmented the cAMP-dependent inhibition of

Table 1. Luminal NHE3 activity in pancreatic ducts from WT and ΔF mice.

Age of mice	Luminal Na^+/H^+ exchange activity (DpH/min)		
	WT/WT	DF/DF	
2 weeks	1.05 ± 0.15	0.29 ± 0.13**	P<0.01
2 months	1.09 ± 0.09	0.25 ± 0.13**	P<0.01
6 months	0.92 ± 0.10	0.38 ± 0.12**	P<0.01

Pancreatic ducts were microdissected from WT and ΔF mice, cannulated and used to measure the luminal Na^+/H^+ exchange activity [3].

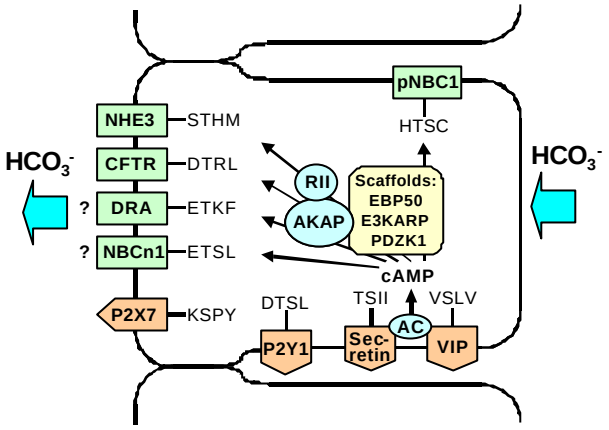

Figure 1. Possible multiple protein interactions controlling HCO_3^- secretion in pancreatic ducts. Several membrane receptors and transporters participating in HCO_3^- homeostasis in pancreatic duct cells have a PDZ-binding motif (-X-T/S-X-hydrophobic amino acid) on their C-terminus. The C-terminal sequences are based on the human clones. Except for several purinergic receptors, most of the proteins are associated with cAMP-dependent processes.
The abbreviations used are: DRA, down-regulated in adenoma; NBC, Na^+-HCO_3^- co-transporter; P2, purinergic receptor; RII, regulatory subunit of protein kinase A type II.

NHE3 in both PS120 cells and pancreatic ducts. Pancreatic ductal fluid and HCO_3^- secretion is stimulated by the G_s-coupled secretin or vasoactive intestinal polypeptide (VIP) receptors. Upon cell stimulation, cellular cAMP is increased and the CFTR-EBP50-NHE3 complex either is formed or may undergo a conformational change to allow regulatory inhibition of Na^+-dependent H^+/OH^- fluxes by CFTR. This inhibits HCO_3^- absorption by the duct cells. At the same time, CFTR stimulates HCO_3^- secretion by activating a Cl^-/HCO_3^- exchange process in the luminal membrane of the pancreatic duct [1, 2]. The overall result is production of an alkaline pancreatic juice.
These findings demonstrate a coordinated regulation of HCO_3^- secretion mediated by the CFTR-NHE3 protein complex. In this respect, it is of particular interests that many of the G protein-coupled membrane receptors and transporters related to HCO_3^- secretion in pancreatic duct cells have a PDZ-binding motif on their C-terminus (Figure 1). In addition, most are associated with cAMP-dependent processes. It has been shown that the scaffolds EBP50 and E3KARP can recruit possible A-kinase anchoring proteins (AKAP) such as ezrin to the protein complex, hence increasing the signaling efficiency of cAMP [8]. Such an arrangement allows for precise and tight control of HCO_3^- homeostasis by CFTR.

Key words Bicarbonates; Cystic Fibrosis Transmembrane Conductance Regulator; Pancreas; Protein Binding; Sodium-Hydrogen Antiporter

Abbreviations AKAP: A-kinase anchoring proteins; CFTR: cystic fibrosis transmembrane conductance regulator; EBP50: ezrin-binding phosphoprotein 50; NHE: Na^+/H^+ exchanger; PDZ: PSD95, Dlg1, ZO-1; VIP: vasoactive intestinal polypeptide; WT: wild type; ΔF: CFTR-impaired homozygote ΔF508

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