Coordination of Pancreatic HCO$_3^-$ Secretion by Protein-Protein Interaction between Membrane Transporters

Min Goo Lee$^1$, Wooin Ahn$^1$, Jin Ah Lee$^1$, Joo Young Kim$^1$, Joo Young Cho$^2$, Orson W Moe$^3$, Sharon L Milgram$^4$, Shmuel Muallem$^2$, Kyung Hwan Kim$^1$

$^1$Department of Pharmacology, Yonsei University College of Medicine. Seoul, Korea. $^2$Department of Physiology and $^3$Department of Internal Medicine, University of Texas, Southwestern Medical Center. Dallas, Texas (USA). $^4$Department of Cell and Molecular Physiology, University of North Carolina. Chapel Hill, North Carolina (USA)

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 important implications 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>
<thead>
<tr>
<th>Age of mice</th>
<th>Luminal Na$^+$/H$^+$ exchange activity (ΔpH/min)</th>
<th>ΔF/ΔF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>1.05 ± 0.15</td>
<td>0.29 ± 0.13**</td>
</tr>
<tr>
<td>2 months</td>
<td>1.09 ± 0.09</td>
<td>0.25 ± 0.13**</td>
</tr>
<tr>
<td>6 months</td>
<td>0.92 ± 0.10</td>
<td>0.38 ± 0.12**</td>
</tr>
</tbody>
</table>

Pancreatic ducts were microdissected from WT and ΔF mice, cannulated and used to measure the luminal Na$^+$/H$^+$ exchange activity [3].
Pancreatic ductal fluid and HCO\textsubscript{3}\textsuperscript{-} secretion is stimulated by the G\textsubscript{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\textsuperscript{+}-dependent H\textsuperscript{+}/OH\textsuperscript{-} fluxes by CFTR. This inhibits HCO\textsubscript{3}\textsuperscript{-} absorption by the duct cells. At the same time, CFTR stimulates HCO\textsubscript{3}\textsuperscript{-} secretion by activating a Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} 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\textsuperscript{+}-HCO\textsubscript{3}\textsuperscript{-} co-transporter; PDZ: PSD95, Dlg1, ZO-1; VIP: vasoactive intestinal polypeptide; WT: wild type; ΔF: CFTR-impaired homozygote ΔF508

**Acknowledgments** This work was supported by the Brain Korea 21 Project for Medical Sciences, Yonsei University (K.H.K.) and the Korean Medical Association in the program year of 2000 (W.A.).

**Correspondence**
Min Goo Lee
Department of Pharmacology
Yonsei University College of Medicine
134 Sinchon-dong
Seoul 120-752
Korea
Phone: +82-2-361.5221
Fax: +82-2-313.1894
E-mail address: mlee@yumc.yonsei.ac.kr

**References**
1. Lee MG, Wigley WC, Zeng W, Noel LE, Marino CR, Thomas PJ, Muallem S. Regulation of Cl-/ HCO3-


