Role of PDZ Domain-Containing Proteins and ERM Proteins in Regulation of Renal Function and Dysfunction

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Hormonal, dietary, and metabolic factors play an important role in regulation of renal proximal tubular apical membrane sodium-hydrogen (Na/H) exchange and sodium-phosphate (Na/Pi) cotransport and basolateral membrane sodium-potassium-ATPase (Na-K-ATPase) activity by diverse cellular mechanisms including transcriptional, translational, and posttranslational mechanisms. Recent work in this area indicates novel protein interactions that play an important role in the fine regulation of these important physiologic processes.

Parathyroid hormone (PTH) is well known to cause rapid inhibition of Na/H exchange activity and Na/Pi cotransport activity by inducing endocytic internalization of Na/H exchange protein (NHE3) (1) and Na/Pi cotransport protein (NaPi IIa) (2) from the apical brush border membrane. PTH also causes rapid inhibition of Na-K-ATPase activity and endocytic internalization of Na-K-ATPase protein (3) from the basolateral membrane. PTH activates a number of protein kinases including protein kinase A (PKA), C (PKC), G (PKG), and ERK/MAPK kinase. Although PTH and/or the signaling intermediates that activate these protein kinases have been shown to induce phosphorylation of NHE3 and Na-K-ATPase before or in parallel with the inhibition of their activity (1,3), it has not been possible to date to demonstrate the direct phosphorylation of NaPi IIa (4). This has raised the possibility of closely interacting regulatory proteins that may be a target of PTH and regulate NaPi IIa independent of direct phosphorylation. Indeed biochemical and molecular (yeast two-hybrid) approaches have led to the identification of two novel classes of signaling complexes, including NHERF-1, NHERF-2, PDZK1, CAL, and ZO-1 have been identified in the kidney that play an important role in the following: (a) the regulation of the expression and activity of renal proximal tubular brush border membrane transport proteins, including NHE-3 (5) and NaPi IIa (4), basolateral membrane transport proteins, including Na-K-ATPase, and Na-HCO3 cotransporter (NBC) (8); (b) the regulation of the expression and activity of cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel and channel regulator (9–13); (c) PTH 1 receptor signaling (14) as well as endocytic sorting of the α2-adrenergic receptor (15) and platelet-derived growth factor receptor (PDGFR) (16,17); (d) epithelial cell polarity and formation of tight junctions (18,19); and (e) maintaining the integrity of the glomerular barrier to proteins through interactions with podocalyxin, negatively charged sialoprotein expressed on the surface of the glomerular visceral epithelial cells or podocytes (20–25).

In addition to their interaction with membrane proteins and receptors, the PDZ domain-containing proteins also interact with F-actin cytoskeleton through their interactions with the ezrin-radixin-moesin (ERM) proteins (26). ERM proteins are typically located peripherally in the membrane and link the cytoplasmic tails of membrane proteins and receptors to the cortical actin cytoskeleton. The ERM proteins play an important role in the formation of microvilli, cell-cell junctions, and membrane ruffles and also participate in signal transduction pathways. The ERM proteins contain an F-actin binding site within their carboxy-terminal 30 residues. In addition, the ERM proteins have a FERM (four-point one, ezrin, radixin, moesin) domain, which are generally located at or near the amino terminal and act as multifunctional protein and lipid binding sites. The FERM domain of ezrin interacts strongly with NHERF-1 and NHERF-2. NHERF-1 and NHERF-2 have two PDZ domains and have a carboxy-terminal sequence of 30 amino acids that bind ezrin.

NHERF-1 (the sodium-hydrogen exchanger regulatory factor-1, also called EBP50) was isolated initially as a co-factor necessary in PTH and cAMP-induced inhibition of NHE3 (27–29). A second member of this family NHERF-2 (also called E3KARP) was also subsequently cloned. NHERF-1 and NHERF-2 are docking proteins that assemble multi-protein signaling complexes, including ezrin, NHE3, and PKA, through their multiple PDZ domains. These protein-signaling...
complexes facilitate the phosphorylation of NHE3 by PKA, resulting in inhibition of NHE3 activity. The significance of this interaction has been demonstrated in opossum kidney (OK) cells expressing an ezrin-binding domain–deficient truncation of NHERF-1, which results in disruption of the association between NHERF-1 and NHE3 and a loss of regulation of NHE3 by PTH and cAMP (30). NHERF-1 therefore plays a critical role in the regulation of renal acidification and sodium transport by PTH and presumably other regulatory hormones.

Using the molecular approach (yeast two-hybrid), several proteins with PDZ domains that interact with NaPi IIa have been identified. Proteins that interact with the C terminus of NaPi IIa include the PDZ proteins NaPi-Cap1 (the mouse ortholog of the human PDZK1), NaPi-Cap2, and NHERF-1 (4,31,32). Both NaPi-Cap1 and NHERF-1 are localized in the renal proximal tubule apical brush border membrane, whereas NaPi-Cap2 is predominantly localized in the subapical compartment. NaPi-Cap1 interacts with the PKA-anchoring protein AKAP2 that suggests that NaPi-Cap1 serves as an organizer of the signaling pathway that is involved in regulation of NaPi IIa. The importance of the NaPi IIa interaction with NHERF-1 was shown in a recent study in which targeted disruption of the mouse NHERF-1 gene was associated with decreased brush border membrane expression and increased intracellular localization of NaPi IIa resulting in decreased renal phosphate reabsorption and renal phosphate wasting (33). On the other hand, targeted disruption of NHERF-1 did not modulate the brush border membrane expression and localization of NHE3. Furthermore, targeted disruption of the PDZK1 gene also failed to modulate the brush border membrane expression of NaPi IIa (34). NHERF-1 therefore plays a critical and unique role in the renal proximal tubular apical membrane targeting of NaPi IIa protein and maintenance of phosphate homeostasis.

Several PDZ domain-containing proteins, including NHERF-1, NHERF-2, and CAP70, bind to the C terminal amino acids of CFTR (cystic fibrosis transmembrane conductance regulator) (9–13). CAP70 and NHERF-1 anchors CFTR to the cytoskeleton at a subapical compartment targeting protein kinase A near CFTR. In addition, CAP70 and NHERF-1 enhance apical membrane CFTR chloride channel activity by cross-linking CFTR dimers. A recent study has identified an additional CFTR-associating, PDZ domain containing protein, CAL (CFTR-associated ligand) (35). CAL favors retention of CFTR within the cell, similar to what occurs in cystic fibrosis, whereas NHERF-1 favors surface expression of CFTR by competing with CAL for the binding of CFTR. Thus PDZ-binding domains tightly regulate the proper cellular trafficking and function of CFTR.

Podocalyxin is the major sialoprotein of glomerular epithelial cells (podocytes) and helps maintain the characteristic architecture of the foot processes and the patency of the filtration slits that determine the glomerular permselectivity to size and charge. Podocalyxin is linked to ezrin and the actin cytoskeleton via NHERF-2 and the podocalyxin/NHERF-2/ezrin multimeric complex is localized along the apical domain of the podocyte plasma membrane (20–22). The podocalyxin/NHERF-2/ezrin/actin complex is disrupted in podocytes studied from puromycin aminonucleoside (PAN), protamine sulfate, or sialidase-treated rats, conditions that result in a dramatic loss of foot processes and a marked increase in urinary protein excretion, comparable to that seen in nephrotic syndrome (20). PDZ-binding domains are therefore also critical in maintaining the glomerular cell integrity and permselectivity and avoiding glomerular protein leak.

In the current issue of JASN, Lederer et al. further explore the potential role of NHERF-1 in regulation of Na-K-ATPase activity and NaPi cotransport activity by PTH. To this end the authors expressed wild-type murine NHERF-1 or mNHERF-1 lacking the ezrin-binding domain in OK cells. They found that PTH inhibited Na-K-ATPase activity in OK cells expressing wild-type mNHERF-1, which was associated with increased serine phosphorylation of the alpha subunit of Na-K-ATPase. In contrast in OK cells expressing mNHERF-1 lacking the ezrin-binding domain, the serine phosphorylation of the alpha subunit of Na-K-ATPase was diminished and the activity of Na-K-ATPase was increased in response to PTH. This study documented for the first time the potential role of NHERF-1/ezrin in the regulation of Na-K-ATPase activity by PTH.

Furthermore, the authors found that in OK cells expressing mNHERF-1 lacking the ezrin-binding domain basal NaPi cotransport activity and apical membrane NaPi IIa protein expression were decreased compared with OK cells expressing wild-type mNHERF-1. However, compared with OK cells expressing wild-type mNHERF-1, expression of mNHERF-1 lacking the ezrin-binding domain had no significant effect on the inhibition of NaPi cotransport activity and NaPi IIa protein expression level in response to PTH or activators of PKA and PKC. These experiments suggest that although NHERF-1 is important for the apical membrane targeting and expression of NaPi IIa protein, the mechanism for endocytosis of NaPi IIa from the apical membrane in response to PTH/PKA/PKC does not require NHERF-1. The cellular mechanism by which PTH induces NaPi IIa internalization is therefore still in need of additional investigation.

The PDZ domain-containing proteins, NHERF-1, NHERF-2, PDZK1, CAL, and ZO-1, and the ERM protein, ezrin, therefore play an important role in kidney physiology, including maintenance of the integrity of the glomerular epithelial cell barrier to proteins, regulation of renal proximal tubular phosphate reabsorption, sodium reabsorption, and hydrogen ion secretion. These protein-protein interactions may offer several novel approaches to treatment of pathophysiologic states associated with altered renal phosphate, sodium, and hydrogen ion homeostasis.

References


11. Liedtke CM, Yun CH, Kyle N, Wang D: Protein kinase C


See related article, “Role of NHERF-1 in Regulation of the Activity of Na-K ATPase and Sodium-Phosphate Co-transport in Epithelial Cells,” on pages 1711–1719.