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Renal phosphate transporters

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Abstract

Purpose of review—Phosphate homeostasis is tightly controlled by the coordinated activity of bone, kidney, intestine, and parathyroid gland. The renal phosphate transporters have emerged as key regulators of both total body phosphate homeostasis and serum phosphate concentration. This review focuses on the latest updates in phosphate transport and transporters with an emphasis on renal phosphate transporters.

Recent findings—Structure function analysis of type II sodium phosphate cotransporters has revealed motifs with significant similarity to those seen in other sodium-coupled solute transporters, identifying key amino acid residues important for solute binding and transport. Previously unidentified regulators of these transporters have been found, although their physiologic significance and interaction with more traditional regulators have not been established. Type II and type III sodium phosphate cotransporters play critical roles in bone, choroid plexus, and vascular physiology and pathophysiology.

Summary—Increasing knowledge of structure function relationships for sodium phosphate cotransporters, as well as greater appreciation for the complexity of their regulation and role in renal and nonrenal tissue, brings the promise of newer, more specific treatments for disorders of phosphate homeostasis.

Keywords

epithelial transport; membrane trafficking; PDZ domain; phosphate homeostasis; sodium phosphate cotransporter; vascular calcification

INTRODUCTION

Phosphate homeostasis is maintained by the coordinated actions of the parathyroid gland, bone, intestine, and kidney. Because renal excretion of phosphate is the final step regulating

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Conflicts of interest

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total body phosphate homeostasis, research has focused on understanding renal phosphate handling. These studies over the past four decades have demonstrated that proximal tubule reabsorption of filtered phosphate is a key regulatory process, mediated by at least three different sodium-dependent phosphate transporters: the type IIa sodium phosphate cotransporter (Npt2a, NaPi-IIa, SLC34A1), the type IIc sodium phosphate cotransporter (Npt2c, NaPi-IIc, SLC34A3), and the type III sodium phosphate cotransporter Pit-2 (Ram-1, SLC20A2). In mouse, in which the most extensive work has been performed, the bulk of the proximal tubule phosphate reabsorption, 70–80%, is mediated by Npt2a, followed by Npt2c with a small contribution by Pit-2. Absence of Npt2a results in a severe phosphate-wasting phenotype with the development of osteomalacia, hypervitaminosis D, hypercalciuria, and kidney stones. Deletion of Npt2c, globally or in kidney alone, results in a much milder phenotype [1]. The contribution of Pit-2 remains unclear. The situation in human beings is quite different. Mutations in Npt2c result in the syndrome of hereditary hypophosphatemic rickets with hypercalciuria (HHRH), a distinct clinical entity characterized by hypophosphatemia, renal phosphate wasting, hypercalciuria, and rickets. In contrast, with the exception of one study, mutations or polymorphisms in human Npt2a have been associated with kidney stone development and an increased risk of osteoporosis, a much milder phenotype than HHRH. Although all three transporters show some regulation by dietary phosphate, only the type II transporters show regulation by the traditional hormonal regulators of renal phosphate transport, such as parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), or dopamine. The remaining member of the type II family, Npt2b, is widely expressed in many epithelial tissues and mediates approximately 50% of intestinal phosphate transport. Pit-1 (Glv-1, SLC20A1) and Pit-2 are widely expressed on most cells and are thought to serve a housekeeping function in providing a means for most cells to transport phosphate intracellularly [2].

TYPE II SODIUM PHOSPHATE COTRANSPORTERS: STRUCTURE FUNCTION RELATIONSHIPS

Because the type II transporters are clearly regulated and account for the vast majority of renal phosphate transport, these proteins have been extensively studied. A major barrier to understanding their function is the lack of knowledge of their three-dimensional structure. Recently, Fenollar-Ferrer *et al.* [3^{***}] developed a structural model of Npt2a using computational methodology and functional assays of mutated constructs. They identified the sodium-dicarboxylate transporter (NaDC) from *Vibrio cholerae* as a suitable template from which to develop their structural model. Hydropathy analysis showed two repeat units and a conserved QSSS motif similar to the binding motif of NaDC. On the basis of this model, they identified potential specific substrate coordination sites, mutated the identified amino acids, and confirmed the importance of these amino acids for the transport function of Npt2a constructs by expressing these mutations in *Xenopus* oocytes and measuring phosphate transport. From these studies, they developed a structural model that will serve as a starting point to develop a more robust understanding of the structure function relationships of the molecule and possibly newer specific directed therapies to regulate the transport function of Npt2a. Of note, although the type II sodium phosphate cotransporters show significant divergence in their amino acid sequences, they also express important structural similarities,

suggesting that the findings from Npt2a may be applied to the development of structural models of both Npt2b and Npt2c.

Two other recently published studies have examined the effect of lithium on the sodium phosphate cotransporter (Npt2b) function [4] and the correlation between conformational changes in Npt2b and charge movements [5]. Substituting lithium for sodium in various ratios revealed that lithium is capable of binding Npt2b and initiating transport, albeit at a much reduced level. This study also confirmed that two sodium ions bind the transporter before phosphate binds. Using fluorescent-labeled reporters, investigators also identified sites involved in conformational changes correlating with five kinetic states for Npt2b. These insights into the structure function relationships will lay the groundwork for developing transporter-specific agents to address human diseases.

TYPE II SODIUM PHOSPHATE COTRANSPORTERS: INTRACELLULAR LOCALIZATION AND TRAFFICKING

These intrinsic membrane proteins, generally localizing to the apical membrane of polarized epithelial cells, mediate transepithelial phosphate transport. However, little is known about the trafficking mechanisms responsible for their intracellular localization. The type II cotransporters express a postsynaptic density protein, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein (PDZ) binding motif at their extreme carboxy termini, and many laboratories have demonstrated C terminal binding to multiple PDZ proteins, including the sodium hydrogen exchanger regulatory factor (NHERF) family of proteins, PDZ-domain protein interacting specifically with TC10 (PIST), and SHANK2 (SH3 and multiple ankyrin repeat domains protein 2). The critical role of NHERF1 in apical membrane localization of Npt2a in proximal tubule has been well described in several laboratories. Giral *et al.* [6] recently showed that Npt2b interacts with NHERF1 but not PDZK1 (also known as NHERF3) in the rat enterocyte using both immunoprecipitation analysis and FLIM-FRET (fluorescence lifetime imaging microscopy – fluorescence resonance energy transfer) studies, that the PDZ binding domain of Npt2b is critical for this interaction, and that NHERF1 but not PDZK1 mediates apical membrane expression of Npt2b. NHERF1-deficient enterocytes were unable to adapt to a low-phosphate diet with an increase in apical membrane Npt2b expression. A role for another PDZ protein, PIST, in type II sodium phosphate cotransporter localization was published by Lanaspá *et al.* [7]. Exposure of renal proximal tubule cells to high phosphate results in downregulation of Npt2a through endocytosis and transport to the lysosome. These investigators demonstrated that some Npt2a molecules are transported to the trans-Golgi network through interaction with PIST. A high-phosphate diet increased Npt2a–PIST interaction and overexpression of PIST in opossum kidney cells, a model of renal proximal tubule, caused retention of Npt2a in the trans-Golgi network and blocked adaptation to low phosphate. Dobrinskikh *et al.* [8] provided evidence that a third PDZ domain protein, SHANK2, also plays a role in Npt2a intracellular localization. These investigators showed that SHANK2 resides in the apical membrane and that SHANK2-deficient cells exhibit decreased apical membrane expression of Npt2a, when compared with wild-type cells, despite equivalent total cellular expression of protein. Predictably, SHANK2-deficient cells failed to adapt to low-phosphate conditions

normally. Additionally, when wild-type cells were treated with high phosphate, Npt2a and SHANK2 exited the apical membrane together and in close association, as demonstrated by FLIM-FRET techniques. Interestingly, the remaining apical membrane Npt2a was not associated with SHANK2, suggesting that endocytosis of Npt2a was dependent on association with SHANK2.

Several other proteins show significant roles in the trafficking of the type II sodium phosphate cotransporters. Loss of myosin VI (Myo6) in intestine is accompanied by increased apical membrane Npt2b despite total cellular Npt2b expression that is equivalent to wild-type cells [9]. The Myo6-deficient cells are unable to respond to high-phosphate conditions by the removal of Npt2b from the membrane. The intermicrovillar region, in which Npt2b is initially trafficked upon exposure to high phosphate, is detached from the actin cytoskeleton in the Myo6-deficient cells. Animals lacking ezrin, one of a family of proteins that links scaffolding proteins including those of the NHERF family to the cytoskeleton, show hypophosphatemia and renal phosphate wasting [10¹¹]. This phosphate-deficient phenotype correlates with decreased apical membrane expression and increased Golgi expression of Npt2a and NHERF1.

In total, these studies suggest a highly orchestrated and coordinated interplay between the PDZ proteins, motor proteins, and the actin cytoskeleton in regulating the expression patterns and therefore the function of type II sodium phosphate cotransporters.

TYPE II SODIUM PHOSPHATE COTRANSPORTERS: REGULATION BY INTRACELLULAR SIGNALING MECHANISMS

Several recent studies have identified or clarified signaling mechanisms responsible for the regulation of sodium phosphate cotransporter function. Gattineni *et al.* [11], using mouse knockdown models, demonstrated that FGF23 regulates renal phosphate transport through interaction with FGFR1 and FGFR4 receptors. Although it is well known that PTH inhibits phosphate transport and type IIa and IIc cotransporter expression through activation of both protein kinase A and protein kinase C, the specific contribution of each signaling pathway has not been clarified. A study employing a PTH or PTHrP receptor construct lacking the ability to activate phospholipase C revealed the essential role for the phospholipase C-protein kinase C pathway in mediating the sustained hypophosphatemic response to PTH [12¹³]. Mice expressing the mutated receptor showed an initial phosphaturia that reversed after 2 days of PTH infusion. PTH infusion in wild-type mice resulted in a 50% reduction in Npt2a protein and mRNA expression, an effect not seen in the mice expressing the mutated receptor. The absence of PTH-stimulated phospholipase C had no effect on PTH regulation of vitamin D metabolism, FGF23, and calcium homeostasis.

Two studies from Tübingen, Germany, show preliminary evidence for a role for B-RAF and adenosine monophosphate activated kinase (AMPK) in the regulation of phosphate transport [13,14]. Using a *Xenopus* oocyte expression model, the investigators showed that B-RAF increased the current produced by injection of Npt2a or Npt2b mRNA into the oocytes, with an increase in both protein expression and affinity. The mechanism for the increased affinity was not explored, but this report may be the first describing enhanced transport through

changes in affinity for phosphate. Using similar methodology, AMPK was shown to decrease Npt2a mRNA-induced current but had no effect on affinity of phosphate for the transporter.

A very interesting study of the effect of sirolimus on phosphate homeostasis was initiated in response to the phenomenon of post-transplant hypophosphatemia [15¹¹]. Sirolimus given to rats produced hypokalemia, hypophosphatemia, and hyperglycemia along with an increase in urine phosphate excretion. Sirolimus-treated rats had lower PTH but similar FGF23 levels. Expression of Npt2c mRNA was slightly lower in the sirolimus group, but the expression of Npt2a and Pit-2 and phosphate uptake in isolated brush border membrane vesicles was unchanged. The authors speculated that sirolimus might affect either basolateral exit of phosphate or distal nephron phosphate excretion; the two areas of renal phosphate handling that have been largely ignored over the past 20 years.

In another study in which sodium phosphate cotransporter expression and function were ostensibly dissociated, investigators found that goats fed a low-nitrogen diet showed increased Npt2a expression and a corresponding decrease in PTH receptor expression in the kidney, but no increase in brush border membrane vesicle uptake of phosphate [16]. The Npt2a expression was measured in crude membrane preparations and brush border membrane preparations, confirming the increased apical membrane expression.

Most previous studies have demonstrated a relatively close correlation between sodium phosphate cotransporter expression and function. These studies address the need to re-examine this assumption and investigate additional aspects of renal phosphate handling.

TYPE II SODIUM PHOSPHATE COTRANSPORTERS: OTHER TISSUES

Wang *et al.* [17¹²] published a study examining the role of NHERF1 in regulation of PTH-mediated phosphate transport in osteoblasts. Osteoblasts express both Npt2a and Npt2b. Interestingly, the effect of PTH differed between proliferating and differentiated osteoblasts and was dependent on the presence of NHERF1. PTH decreased Npt2a expression and function in proliferating osteoblasts but increased Npt2a expression in differentiated osteoblasts. This effect correlated with an increase in PTH-stimulated ATP release that was NHERF1-dependent and critical for the process of mineralization. This study is one of a few to begin to examine how PTH actions are coordinated between tissues responsible for mineral metabolism.

TYPE II SODIUM PHOSPHATE COTRANSPORTERS

A variety of roles for these transporters, aside from simple provision of phosphate for intracellular needs, are emerging. A previously discussed study highlighted the role of type II sodium phosphate cotransporters in mineralization. Another study shows that Pit-1 and Pit-2 are implicated in osteoinduction stimulated by NELL-1 (expressed in neural tissues and containing EGF-like domains homologue 1) in preosteoblasts [18]. How the roles of these cotransporters are coordinated remains unknown. Evidence suggests that Pit-2 is responsible for transporting phosphate out of the cerebrospinal fluid by the choroid plexus [19]. Both Pit-1 and Pit-2 are expressed, but Pit-1 was found primarily in the vascular

endothelial cells, whereas Pit-2 was seen in the apical microvilli. Phosphate transport was not blocked by 1 mM phosphonoformic acid, a relatively nonspecific phosphate transport inhibitor that has a lesser effect on type III transporters, but was blocked by arsenate, ouabain, sodium-free medium and high potassium. Mutations in type III transporters have been implicated in familial basal ganglia calcifications. The presence of Pit-1 and Pit-2 in normal mouse brain was confirmed in another study [20]. Pit-1 and Pit-2 may have redundant roles in vascular calcification [21] and bone mineralization [22], according to two recent reports showing enhanced Pit-2 expression under conditions in which Pit-1 expression is compromised. However, the functions of the two proteins do not entirely overlap as evidenced by studies showing severe defects in erythroid differentiation in Pit-1-deficient animals [23].

The role of Pit-1 in the phenomenon of vascular calcification continues to be an area of active investigation. Prior studies had demonstrated that phosphate-stimulated calcification in smooth muscle cells was associated with Pit-1-mediated phosphate uptake followed by activation of an osteoblast-like transformation of the cell. Recent studies shed some light on potential mechanisms. El Husseini *et al.* [24] showed that blocking Pit-1 expression blunted phosphate-induced vascular calcification and that Pit-1 expression in human calcified aortic valve tissue was increased. The investigators showed in this study that high phosphate treatment of vascular smooth muscle was associated with low levels of Akt-1, an effect that was blocked by phosphonoformic acid or Pit-1 knockdown. In another study, investigators demonstrated that aldosterone increased Pit-1 mRNA expression, alkaline phosphatase activity, and expression of osteoblast transcription factors such as CBFA1 in human aortic smooth muscle cells [25]. Interestingly, this effect was partially inhibited by FGF23 treatment. Conversely, spironolactone decreased the vascular calcification seen in klotho-deficient mice. These studies begin the search for the pathways mediating the phenomenon of vascular calcification and suggest new therapeutic targets.

CONCLUSION

The type II sodium phosphate cotransporters have fundamental roles in the regulation of total body phosphate homeostasis, predominantly through mediating intestinal and kidney transport but with evidence for an emerging role in bone. The many roles of type III sodium phosphate cotransporters in a wide variety of organ systems are only now beginning to be appreciated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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▪ of special interest

▪▪ of outstanding interest

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KEY POINTS

- Type II sodium phosphate cotransporters play major roles in the regulation of total body phosphate homeostasis through mediating intestinal and kidney phosphate transport.
- Type III sodium phosphate cotransporters have widespread and poorly understood functions but appear to play a major role in pathologic calcification.
- Both types of transporters contribute to normal bone mineralization.
- Recent studies are uncovering important structure function correlations.