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Intestinal Phosphate Transport

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Abstract

Phosphate is absorbed in the small intestine by at least two distinct mechanisms: paracellular phosphate transport which is dependent on passive diffusion and active transport which occurs through the sodium-dependent phosphate co-transporters. Despite evidence emerging for other ions, regulation of the phosphate specific paracellular pathways remains largely unexplored. In contrast, there is a growing body of evidence that active transport through the sodium-dependent phosphate co-transporter Npt2b is highly regulated by a diverse set of hormones and dietary conditions. Furthermore, conditional knockout of Npt2b suggests that it plays an important role in maintenance of phosphate homeostasis by coordinating intestinal phosphate absorption with renal phosphate reabsorption. The knockout mouse also suggests that Npt2b is responsible for the majority of sodium-dependent phosphate uptake. The type III sodium-dependent phosphate transporters, Pit1 and Pit2 contribute a minor role in total phosphate uptake. Despite co-expression along the apical membrane, differential responses of Pit1 and Npt2b regulation to chronic versus dietary changes illustrates another layer of phosphate transport control. Finally, a major problem in chronic kidney disease (CKD) patients is management of hyperphosphatemia. The present evidence suggests that targeting key regulatory transporters of intestinal phosphate transport may provide novel therapeutic approaches for CKD patients.

Introduction

Systemic phosphate homeostasis is balanced through three major mechanisms: intestinal uptake, retention or release from bone, and regulated renal reabsorption. Under typical dietary conditions, the vast majority of phosphate is absorbed through the intestine, and systemic balance is primarily regulated through changes in fractional excretion of phosphate.(1-3) Recent studies suggest that the intestine plays a more active role in phosphate homeostasis than previously appreciated through its ability to couple phosphate uptake with release of hormones that signal phosphate changes in the kidney and the bone. (4-7) Accumulating evidence indicates that there is remarkable diversity in both the types and regulation of intestinal phosphate absorption. Current research is focused on the relationship of these diverse mechanisms and systemic phosphate control in normal physiology and during chronic kidney disease. This chapter will focus on our current understanding of the types and regulation of intestinal transport mechanisms.

Disclosures: Yves Sabbagh and Susan Schiavi are employees of Genzyme Corporation and own stock in the company.

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Physiology of Intestinal Phosphate Absorption

Inorganic phosphate is absorbed along the entire length of the intestine, with the small intestine having a significantly higher absorption capacity compared to the colon.(8-15) Phosphate absorption in the colon seems to be physiologically irrelevant under most settings and is observed only under conditions of extremely high luminal phosphate concentrations, such as through the use of phosphate enemas that increase the luminal phosphate to greater than 1500 times the blood concentration.(15-17)

Classic studies performed over a half century ago led to the current dogma that intestinal phosphate transport occurs through two distinct mechanisms, a non-active and a sodium-dependent transport pathway. Pioneering experiments performed by McHardy and Parsons assessed the relative absorption rates by monitoring temporal changes in serum phosphate levels after gavaging solutions containing inorganic phosphate at concentrations ranging from 12.5 to 100 mM.(8) These seminal studies pointed to a passive diffusion mechanism since the rates of phosphate absorption directly correlated with luminal phosphate concentrations without apparent saturation. However, the rate of phosphate absorption was significantly decreased in the presence of a low sodium concentration providing the first line of evidence for a sodium-dependent pathway.

Subsequent *in vivo* studies used radio-labeled tracers to confirm both the existence and the relative contribution of these two distinct pathways.(18,19) *In vitro* studies utilizing Ussing chambers, voltage clamp analysis, or radiolabel uptake in intestinal segments or in brush border membranes under conditions that eliminated the electrochemical gradient validated the presence of an active sodium-dependent transport mechanism.(20-22) Moreover, when these assays were performed under conditions where concentration gradients were established, the existence of a non-sodium, non-energy dependent transport pathway was also confirmed. Subsequent experimental emphasis focused on the relative contribution of each pathway with results varying widely with active transport estimated to be anywhere between 30 and 80% of total transport.(7,8,20,21,23) Although the discrepancy between these results can be attributed to differential sensitivities of individual techniques, it is clear that the overall estimate of active versus passive transport is highly dependent on the absolute phosphate concentrations which dictate the level of non-active transport. Taken together, these studies revealed that phosphate is absorbed through a minimum of two distinct mechanisms.

Paracellular Phosphate Transport

Passive transport mechanisms generally depend on electrochemical gradients across an epithelial layer with actual paracellular movement occurring through tight junction complexes that are formed by the interaction of complementary adhesive proteins of neighboring cells. An emerging body of literature demonstrates that these tight junctions are regulated by signal transduction pathways, actively interact with cytoskeleton, and have specificity toward individual or selected ion groups.(24-28) Two major components of tight junctions, occludins and claudins appear important for ion specificity. This is illustrated by the finding that mutations in claudin 16, a key molecule within tight junctions of the intestine results in hypomagnesemia with hypercalceruria.(24-26) Despite the growing understanding of how cellular regulatory mechanisms can influence passive transport, specificity for phosphate in association with specific tight junction proteins has not been reported.

Sodium-Dependent Phosphate Co-transport

Two families of sodium dependent phosphate transporters, type II (SLCA34) and type III (SLC20), are responsible for the inward transport of extracellular phosphate.(29-31) The type III transporters which include Pit1 and Pit2 are expressed broadly across a variety of cell types and are generally considered to be involved in supplying cells with inorganic phosphorus to meet the needs of individual cell functions.(32) The type II sodium dependent phosphate transporters appear to be the major handlers of sodium dependent phosphate in the kidney (Npt2a and Npt2c) and the intestine (Npt2b). The vast majority of studies on phosphate transport have centered on the critical role of Npt2a and the more recently discovered Npt2c in management of systemic phosphate homeostasis.

The pivotal discovery of the type-II sodium-dependent phosphate transporter, Npt2b in lung and small intestine has formed the basis for an emerging understanding of intestinal phosphate transport mechanisms.(33) Similar to Npt2a, Npt2b is electrogenic and transports phosphate with a stoichiometry of 3:1 $\text{Na}^+:\text{HPO}_4^{2-}$ with a relative K_m of $\sim 10 \mu\text{M}$ as defined by kinetic studies of Npt2b expressed in *Xenopus laevis*.(34,35)

The relative high affinity of phosphate for this transporter has lead to the suggestion that Npt2b can be saturated under most dietary conditions and therefore might be expected to be a major contributor only under conditions of dietary phosphate depletion. As discussed below, studies utilizing a conditional knockout mouse have demonstrated that Npt2b can play a significant role in both intestinal phosphate sensing and transport.

Regulation of Intestinal Phosphate Transport

A variety of factors have been shown to specifically or indirectly modulate Npt2b expression and/or sodium-dependent phosphate transport in the intestine. These include epidermal growth factor (EGF), thyroid hormone, glucocorticoids, estrogens, metabolic acidosis, matrix extracellular phosphoglycoprotein (MEPE) and fibroblast growth factor 23 (FGF23).(36-46) Several of these factors including FGF23 and MEPE, proteins associated with the phosphatonin pathway, have also been shown to modify renal phosphate transport. The relative role of these diverse factors in relationship to each other and in normal physiology will require additional studies.

In contrast, several studies have investigated the mechanisms of Npt2b regulation by the two major stimulators of Npt2b; dietary phosphate and $1,25(\text{OH})_2\text{D}_3$. (36,47) These experiments reveal diversity in the cellular mechanisms controlling active phosphate transport. Because $1,25(\text{OH})_2\text{D}_3$ is typically induced in response to hypophosphatemia, it was originally assumed that both pathways ultimately activated the nuclear vitamin D receptor (VDR) to stimulate Npt2b transcription. However, directed studies challenged this assumption by demonstrating that low phosphate diets induced Npt2b protein and transport activity without apparent changes in mRNA expression.(47,48) Subsequent studies utilized VDR knockout mice with hypophosphatemia as a result of elevated PTH levels demonstrated that $1,25(\text{OH})_2\text{D}_3$ had no influence on sodium phosphate transport or Npt2b expression.(49) In contrast, administration of a low phosphate diet significantly increased Npt2b protein expression in both VDR knockout and wild-type mice suggesting that the intestinal sodium phosphate co-transport adaptation occurs independently of $1,25(\text{OH})_2\text{D}_3$. Thus, regulation of $1,25(\text{OH})_2\text{D}_3$ action appears to be a transcription dependent pathway involving the VDR, whereas low dietary phosphate enhances sodium dependent phosphate transport through a post-transcriptional mechanism.(49,50) These studies clearly demonstrate that Npt2b regulation by either low dietary phosphate or increased $1,25(\text{OH})_2\text{D}_3$ can occur via distinct pathways.

The specific post-transcriptional mechanisms involved in regulation of Npt2b are not entirely known. However, a post-transcriptional mechanism has been described for glucocorticoids and serum factors involving the actions of serum and glucocorticoid-inducible kinase 1 (SGK1). This pathway has been shown to enhance Npt2b expression by inhibiting protein degradation. Similar to Npt2b, SGK1 is expressed in the villi of enterocytes at the apical side and is absent in crypt cells.(51) When co-expressed with Npt2b in *Xenopus laevis* oocytes SGK1 phosphorylates and inactivates Nedd4-2, a ubiquitin ligase that stimulates Npt2b activity by preventing its protein degradation.(52) Additional structures within Npt2b appear to be important for appropriate cellular localization as a carboxy terminal stretch of cysteines is responsible for preventing its insertion into the basolateral membrane.(53)

It is important to note that despite strong homology, distinct regulatory mechanisms have evolved for Npt2b and Npt2a. For example, parathyroid hormone (PTH), mediates the retrieval and subsequent degradation of Npt2a while it has no effect on Npt2b protein, perhaps due to the requirement of a dibasic motif that is absent in Npt2b.(54)

The Role of Npt2b

The relative diversity in Npt2b regulatory mechanisms suggests that Npt2b may be an important component of phosphate homeostasis. Consistent with this hypothesis, constitutive Npt2b deletion in mice has an embryonic lethal phenotype.(55) To fully understand the function of NPT2b in intestinal phosphate absorption, a non-developmental conditional Npt2b KO mouse was generated that eliminated Npt2b protein in all tissues in adult animals.(7) Despite clear evidence of decreased intestinal phosphate absorption in the Npt2b KO mouse, serum phosphorus and calcium concentrations were maintained in these animals due to compensatory upregulation of the renal sodium phosphate cotransporter Npt2a. The increased renal phosphate reabsorption could be explained by reduced levels of the phosphaturic hormone FGF23, which led to a concomitant elevation of serum 1,25(OH)₂D₃ and subsequent up-regulation of Npt2a.(7) These results imply that Npt2b may be part of the machinery regulating hormonal changes to maintain systemic phosphate handling.

The findings in the knockout mouse may explain the observations that inactivating *NPT2b* mutations in the human population do not have net changes in serum or urinary calcium and phosphate levels despite the presence of pulmonary alveolar microlithiasis (calcium phosphate deposits in their lungs).(56,57) Although not yet reported, it would be interesting to determine if FGF23 values are depressed relative to the general population similar to observations described in the knockout mice.

The availability of the Npt2b KO mice also provided a means to assess the contribution of Npt2b-dependent phosphate transport relative to passive transport. To model phosphate absorption under postprandial conditions, an acute model of hyperphosphatemia was used to maximize the contributions of both passive and active transport. These studies revealed that Npt2b-dependent phosphate transport contributes as much as 45 to 50% of total phosphate transport in the first hour after a phosphate bolus.(7) In addition, studies using the everted sac method to measure phosphate transport in a segment of the small intestine *ex vivo* showed that under the conditions tested, Npt2b accounted for more than 90% of sodium-dependent transport.(7) Taken together, the Npt2b knockout mouse underscores the role of Npt2b in phosphate homeostasis and further demonstrates that organ cross-talk between the intestine, kidney and bone regulates the FGF23/1,25(OH)₂D₃ hormonal axis.

The above studies did not rule out the presence of other intestinal phosphate transporters since approximately 10% of the sodium-dependent transport could not be attributed to

Npt2b. Recent evidence suggests that the type III sodium-dependent transporter, Pit1 also contributes to overall regulation. Although it has been widely accepted that Pit1 and Pit2 are ubiquitously expressed, acting as housekeeping transporters at the basolateral membrane, new data demonstrates that Pit1 protein is actually expressed in the apical membrane of enterocytes.(47,58) Npt2b and Pit1 proteins are mostly expressed in the duodenum and jejunum of rat small intestine; while their expression is negligible in the ileum. In contrast, Npt2b expression in mouse is expressed in jejunum and ileum.(6) To continue understanding the role of Pit1 in overall transport it will be helpful to determine its protein regulation in murine models. Pit2 transporter mRNA and protein have also been detected in the apical membrane of enterocytes but a specific role for this transporter has not yet been defined. (35,58,59)

Despite similarities between Npt2b and Pit1 rat expression patterns, differences in adaptive responses to acute versus chronic changes in dietary phosphate have been identified. To identify and compare regulatory pathways that occur after chronic changes in dietary content over days versus those that happen acutely (after 4 hour feeding) such as after a meal, phosphate transport activity and phosphate transporter protein and mRNA expression were monitored. In these studies, the adaptive response to a chronic low phosphate diet (0.1% phosphate) appears to be restricted to the jejunum, with increased brush border membrane (BBM) sodium-dependent phosphate transport activity and increases in Npt2b, but not Pit1, protein and mRNA abundance.(5) However, in rats acutely switched from a low to a high phosphate diet (1.2% phosphate), there is an increase in BBM sodium-dependent phosphate transport activity in the duodenum that is partially associated with an increase in BBM Npt2b protein abundance with no observed increase in Npt2b mRNA.(5) These observations are consistent with a role of post-translational mechanisms in acute adaptation. Furthermore, the fact that duodenal and jejunal segments show such differential adaptation suggests that distinct regulatory mechanisms exist along the intestinal tract. A similar regional specific regulation has been shown in response to $1,25(\text{OH})_2\text{D}_3$ in rats and mice, where the hormone increases the phosphate absorption rate in the jejunum but does not affect the transport in the duodenum.(6) These region-specific adaptations may reflect the differential expression of yet to be identified regulatory proteins that modify the expression and/or activity of the Npt2b transporter.

The acute adaptive upregulation of Npt2b in the rat duodenum described above can lead to a transient postprandial hyperphosphatemia that can last several hours. Recent studies addressing the adaptive changes in response to different levels of dietary phosphate in human subjects have shown similar postprandial serum phosphate elevations.(60,61) In individuals fed a high phosphate diet, a significant postprandial rise in serum phosphate, with a peak value exceeding the normal range at 2 h, was observed. Although high phosphate diet-induced hyperphosphatemia seems to be milder in humans (from 3.5 to 5.0 mg/dl) than in rats (from 5 to 17 mg/dl), the rat model appears suitable to study postprandial mechanisms that are physiologically meaningful in both species.

Intestinal Phosphate Absorption and Chronic Kidney Disease (CKD)

Hyperphosphatemia due to declining renal function is linked to disease progression, increased vascular stiffness, vascular calcification and increased cardiovascular morbidity and mortality.(62-64) Mechanisms that cause hyperphosphatemia in CKD likely reflect pathological changes in both chronic and acute regulatory pathways.

Although studies in the Npt2b knockout mice demonstrated compensation in renal excretion, there does not appear to be an apparent change in total intestinal phosphate absorption in wild-type or knockout animals with mild uremia.(65) However, some caution should be

used in over-interpretation of these animal studies particularly in models where acute injury has been the primary inducer of CKD and a loss of 1 α -hydroxylase expression is not observed. Interestingly, in a model of adenine-induced chronic kidney disease, the rise in serum phosphate associated with CKD is attenuated in uremic Npt2b-knockout mice compared with uremic wild-type control mice.(66) This also translated to improved survival of the uremic Npt2b knockout mice compared to uremic wild-type mice within the same study.(66)

The important role of Npt2b in mediating phosphate transport is further illustrated in rodent and human studies showing that administration of nicotinamide or nicotinic acid in a chronic kidney disease setting lowers serum phosphate.(67-69) Intestinal brush border membranes prepared from the jejunum of rats treated with nicotinamide resulted in a significant inhibition of sodium-dependent phosphate transport and decreased vitamin D receptor (VDR) mRNA levels.(70) Intestinal absorption of radiolabeled phosphate in adenine-induced CKD rats was attenuated by nicotinamide treatment.(67) Reduced phosphate uptake correlated with decreased Npt2b protein expression.(67) In hemodialysis patients, nicotinamide treatment was associated with significantly decreased serum phosphorus and serum iPTH.(69) A reduction in both calcium phosphate product and serum phosphate was observed in hemodialysis patients treated with nicotinic acid analogues. (68,71,72) Finally, a recent study has shown that dyslipidemic CKD patients in stages 1 to 3 treated with extended release niacin have sustained reduction in serum phosphate levels.(73) Taken together, these results illustrate the important contribution of Npt2b to phosphate absorption.

In addition to chronic changes, postprandial changes in serum phosphate have also been associated with increased risk of cardiovascular disease in CKD patients as well as in healthy individuals with normal kidney function.(74-77) In fact, CKD patients could be even more susceptible to the postprandial transitory hyperphosphatemia for two reasons. First, their impaired phosphate renal excretion and limited capacity to modulate renal reabsorption despite severely elevated PTH and FGF23 could extend the peak levels of serum phosphorus longer than in healthy individuals. Second, the more robust response observed in the rat model is probably related to the previous adaptation to low luminal phosphate levels in the intestinal tract. Unlike the general population, CKD patients could be in a similar adaptive condition if they are under dietary phosphate restriction and/or phosphate binder treatment.

Summary

Emerging data reveals new concepts implicating the intestine as a major player in the regulation of phosphate homeostasis. Intestinal phosphate transport appears to be coupled to systemic hormonal regulation that can influence phosphate handling at distinct sites such as the kidney. An increased understanding of the molecular mechanisms in the intestine may identify novel therapeutic approaches for the treatment of hyperphosphatemia and vascular calcification in the setting of CKD.

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