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REVIEW

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Regulation of the type IIb sodium-dependent phosphate cotransporter expression in the intestine

Abstract Phosphate (Pi) plays important roles in growth, development, bone mineralization, energy metabolism, nucleic acid synthesis, cell signaling, and acid-base regulation. The rate of intestinal absorption of Pi is a major determinant of Pi homeostasis. The type IIb sodium-dependent Pi cotransporter (NaPi-IIb) is responsible for intestinal Pi absorption. Many physiological factors regulate the rate of Pi absorption via modulating the expression of NaPi-IIb in the intestine. In this review, we summarize the role of these factors in the regulation of NaPi-IIb expression in the intestine.

Keywords NaPi-IIb, expression, intestine, regulation

1 Introduction

Phosphorus and phosphate (Pi) play important roles in a variety of biological processes such as growth, development, cell signaling, nucleic acid synthesis, energy metabolism, membrane function, and bone mineralization. Phosphate is localized primarily in bone matrix (85%), and the remains of the body phosphate is divided into intracellular fluids (ICF) (15%) and extracellular fluids (ECF) ($< 0.5\%$). In ICF, phosphate is a component of nucleotides (DNA and RNA), high-energy molecules (e.g., ATP), and metabolic intermediates. In ECF, phosphate is present in its inorganic form and serves as a buffer for pH. Due to the involvement of Pi in diverse biological processes, decreases in serum Pi concentrations and negative Pi balance can result in serious diseases. Acute decreases in serum Pi concentrations can result in myopathy, cardiac dysfunction, abnormal neutrophil function, platelet dysfunction, and red-cell membrane fragility (Xu et al., 2002; Xu et al., 2003). Chronic serum Pi deficiency results in impaired bone mineralization, rickets, and osteomalacia because the rate of bone matrix mineralization depends on the availability of phosphorus and calcium (Cross et al., 1990). Elevated serum Pi concentrations contribute to the pathogenesis of secondary hyperparathyroidism in patients with chronic renal failure (Radanovic et al., 2005). Therefore, it is critically important for the body to control the phosphate level in blood and maintain the phosphate homeostasis. To understand the phosphate homeostasis, it is important to understand how the body organs handle the phosphate. The small intestine is an important site for phosphate absorption. Early studies showed that Pi transporting through the apical membrane of small intestinal epithelial cells is coupled with sodium. One transporter involved in intestinal Pi absorption is the type IIb sodium dependent Pi cotransporter (NaPi-IIb), which has been cloned from rodents, chicken, and humans. NaPi-IIb expression is modulated by many physiological factors, including diet, hormone, vitamin D, development, and segment.

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2 Identification of NaPi-IIb

The solute carrier SLC34 family comprises three members: NaPi-IIa, NaPi-IIb, and NaPi-IIc. NaPi-IIa is primarily expressed in the bush border membrane (BBM) in the renal proximal tubule and is responsible for Pi reabsorption in the kidney. Beside the kidney, expression of NaPi-IIa has been described in the bone and neurons (Murer et al., 2003; Radanovic et al., 2005). The expression of NaPiIIc is found exclusively in the kidney and is described as a growth-related factor. The NaPi-IIc protein (75 kDa) is localized in apical membranes of proximal tubules of deep nephrons.

NaPi-IIb is identified based on EST clones derived from lung tissue (Feild et al., 1999). The expression of NaPi-IIb mRNA has been detected in a number of tissues such as small intestine, lung, mammary glands, testis, and liver. Fully glycosylated NaPi-IIb is observed as a band of approximately 108 kDa and is localized in brush borders of enterocytes, in the apical pole of alveolar type II cells. However, the physiological roles of NaPi-IIb describing these tissues have not yet been entirely defined. In secreting mammary glands, the role of NaPi-IIb can be envisaged delivering Pi into milk during lactation. So far, several observations have provided evidences that NaPi-IIb is involved in the transcellular flux of phosphate in the small intestine, and NaPi-IIb has been cloned and characterized in the intestine of animals, including rodents, human, rabbit, and chicken. After the heterologous expression of NaPi-IIb in Xenopus laevis oocytes, it has been demonstrated that NaPi-IIb-mediated Pi transport is obligatorily dependent on the presence of sodium and exhibits apparent Km values of 50 $\mu mol \cdot L^{-1}$ for Pi and 40 $mmol \cdot L^{-1}$ for sodium ions. On the basis of these results, it can be concluded that transcellular Na^+-dependent absorption of Pi in the intestine is initiated by the NaPi-IIb.

3 Regulation of NaPi-IIb expression

The regulation of membrane-bound transporter proteins is involved in transcriptional factors at the gene level and also protein translation and protein processing controls. The regulation at the transcriptional level results in changes in gene expression expressed as variations in mRNA abundance, while the regulation of protein translation and processing changes the number of functional proteins on the membrane. These regulations result in changes in the overall transporter protein function. Like other membrane-bound transporter proteins, the regulation of NaPi-IIb expression is also involved in transcriptional factors, translational processing control, and intracellular translocation. The regulators of NaPi-IIb in small intestine are phosphate intake, hormone, epidermal growth factor, development, segment, and metabolic acidosis.

3.1 Dietary Pi intake

It is well known that nutrient substrates are among the most significant factors regulating intestinal digestive enzyme and transport activity. Dietary deprivation of phosphate represents the important physiological regulation of small intestinal Pi absorption. On a functional level, up regulation of small intestinal absorption of phosphate duo to a diet of low Pi contend has been described, occurring in many species (Cross et al., 1990). In all of these studies, it has been established that the adaptive response to a low Pi diet leads to an increased Vmax value of the apically located Na-Pi cotransporter. Until recently, Na-Pi cotransporters expressed in mammalian small intestine have been identified and characterized, and the molecular mechanism of the regulation of small intestinal absorption of Pi by a low-Pi diet has been investigated. Hattenhauer et al. (1999) demonstrated that the NaPi-IIb contransporter expressed in the brush border of enterocytes was up regulated by a low-Pi diet. As the up regulation of NaPi-IIb contransporter did not involve alterations in the amount of NaPi-IIb transcript, the effects of a low-Pi diet seem to be unrelated to the transcriptional control of the transporter gene but may be via nongenomic mechanism. Low-Pi diets result in an increase in the intestinal Na-Pi cotransport activity, a rapid decrease in plasma Pi, activation of renal 1, 25-hydroxylase, and an increase in vitamin D3 levels. Several studies suggest that the adaptation of small intestinal Na-Pi cotransport to a low-Pi diet is regulated by vitamin D3, demonstrating that changes in apical Na^+-dependent Pi cotransport rates but not in the apparent Km value for Pi, respond to different levels of dietary Pi content. However, Segawa et al. (2004) demonstrated that intestinal Na-Pi cotransport adaptation to a low-Pi diet occurred independently of vitamin D. Huber et al. (2002) indicated that at least two different mechanisms were involved in goat intestinal Pi absorption. In the duodenum, there is a proton-dependent sodium-sensitive system that is not influenced by dietary P restriction. In the jejunum, Pi transport is modulated by a sodium-dependent proton-sensitive system, mainly by NaPi cotransporter type IIb. This Pi transport system adapts to dietary P restriction by increasing the transporter capacity due to higher transporter protein expression.

3.2 Hormone

3.2.1 Glucocorticoids

Exogenous glucocorticoids (GCs) administration induces precocious intestinal maturation in the first and second postnatal weeks by modulating gene expression, membrane fluidity, and patterns of protein glycosylation. The GC-induced down-regulation of intestinal Na-Pi transport has been reported (Arima et al., 2002). Arima et al. (2002) demonstrated that glucocorticoids injection reduced Na-Pi uptake (3.4-fold), Na-Pi-IIb protein levels (3.8-fold), and mRNA (3.7-fold) in suckling animals, which indicated that the GC-induced decrease in intestinal Na-Pi transport correlated well with Na-Pi-IIb protein and mRNA level reductions in suckling animals. The parallel decline in mRNA abundance implicated a possible genomic effect of GCs.

3.2.2 Estrogen

Estrogen is an important physiological regulator involved in modulating calcium homeostasis (Feild et al., 1999) not only by the regulation of Ca^{2+} absorption but also by the

maintenance of bone density and the modulation of 1, 25-(OH)2 vitamin D3 synthesis. These observations suggested that the estrogen might play a role in intestinal phosphate absorption. Xu et al. (2003) observed that the estrogen treatment stimulated BBMV Na/Pi uptake and western blot analysis results showed that the estrogen treatment increased NaPi-IIb protein abundance in rat intestine. The increase in BBMV Pi uptake, detected after the estrogen treatment, is most likely related to increasing apical NaPi-IIb protein expression, due to the fact that the estrogen treatment increased both intestinal Na/Pi uptake and NaPi-IIb protein levels to a similar extent. Further studies showed that the estrogen treatment also increased NaPi-IIb mRNA abundance in rats. Taken together, these results suggested that the estrogen could increase the intestinal Pi absorption through its stimulatory effect on NaPi-IIb cotransporter gene expression. To decipher the molecular mechanism of estrogen regulation of intestinal NaPi-IIb gene expression, Xu et al. (2002) used human intestinal epithelial (Caco-2) cells as an *in vitro* model. The results demonstrated that the endogenous NaPi-IIb gene expression was stimulated by the estrogen treatment in Caco-2 cells and that this effect was inhibited by the actinomycin D treatment. The increase in NaPi-IIb mRNA abundance in Caco-2 cells after the estrogen treatment is similar to the increase observed in rats, suggesting that the increase in NaPi-IIb mRNA abundance induced by estrogen was likely involved in the synthesis of new NaPi-IIb mRNA in rats and cells. Furthermore, transfection studies with human NaPi-IIb promoter constructs showed that the estrogen increased the NaPi-IIb gene promoter activity by ~36% in transiently transfected Caco-2 cells. The estrogen treatment increases the intestinal Na/Pi uptake in rats at least partially through increasing the NaPi-IIb mRNA and protein abundance. It is also demonstrated that the estrogen can increase the NaPi-IIb mRNA expression in Caco-2 cells. However, the actinomycin D treatment may block the estrogen-induced increases in NaPi-IIb mRNA expression in Caco-2 cells, in which a transcriptional mechanism is likely involved.

3.2.3 Vitamin D3

Vitamin D3, a steroid hormone, plays a central role in modulating the phosphate homeostasis and Pi uptake by the small intestine (Huber et al., 2002). The active form of vitamin D3 is 1, 25-(OH)2 vitamin D3, which is mainly synthesized in the kidney from 25-(OH) vitamin D3. 1, 25-(OH)2 vitamin D3 binds the vitamin D receptor (VDR) to elicit its effect on the regulation of gene expression. 1, 25-(OH)2 vitamin D3 plays important roles in the calcium and phosphate homeostasis, the regulation of the parathyroid hormone system, the inhibition of cell growth, and the induction of cellular differentiation. Many previous studies showed that the 1, 25-(OH)2 vitamin D3 increased the intestinal Pi absorption through the modulation of Na/Pi absorption (Katai et al., 1999). In adult rodents, this increase is at least partially controlled by the modulation of NaPi-IIb protein expression. Xu et al. (2002) demonstrated the direct regulation of NaPi-IIb gene expression by 1, 25-(OH)2 vitamin D3, showing that the 1, 25-(OH)2 vitamin D3 treatment increased the NaPi-IIb mRNA abundance in suckling rats and RIE cells, as well as the NaPi-IIb gene promoter activity in transfected RIE cells. Because the actinomycin D treatment blocked the 1, 25-(OH)2 vitamin D3-induced increases in NaPi-IIb mRNA expression in RIE cells, a transcriptional mechanism is likely involved. The regulation of small intestinal NaPi-IIb cotransport by a low-Pi diet is assumed to be dependent on the stimulation of the renal 25-hydroxyvitamin-D3-1α-hydroxylase activity and the resulting increase of the serum concentration of 1, 25-(OH)2 vitamin D3. Consequently, an increase of NaPi-IIb mRNA may be explained by a transcriptional regulation that involves the vitamin D receptor (VDR), similar to what was described for calbindin-D9k. However, an involvement of VDR in the regulation of NaPi-IIb in intestine by low-Pi diet has recently been challenged because of the unaltered up regulation of the NaPi-IIb protein and mRNA in the VDR-deficient mice compared with wild-type mice (Segawa et al., 2004). Further studies will focus on the identification of the responsive region in the promoter and the trans-acting factors involved in the regulation of the NaPi-IIb gene by 1, 25-(OH)2 vitamin D3.

3.3 Epidermal growth factor

EGF plays an important role in many physiological and pathophysiological processes, such as cell growth and recovery from injury (Trump and Berezesky, 1995). The EGF treatment decreased NaPi-IIb mRNA abundance in rat intestine and in human intestinal cells and also reduced the NaPi-IIb gene promoter activity in transfected human intestinal cells. The actinomycin D treatment blocked the promoter activity decrease induced by the EGF treatment, confirming the transcriptional regulation mechanism. These novel findings suggest that the transcriptional mechanism is involved in the EGF regulation of intestinal NaPi-IIb cotransporter gene expression. Furthermore, a putative EGF response element(s) was shown to be in the region of -1103 bp to -380 bp of the human NaPi-IIb (hNaPi-IIb) gene promoter. Further studies will focus on the identification of the EGF responsive element(s) and the transcription factors involved in the EFG regulation of the human NaPi-IIb gene. Xu et al. (2005) recently cloned the human NaPi-IIb (hNaPi-IIb) cotransporter gene promoter and characterized its regulation by EGF in human intestinal Caco-2 cells. Approximately, 2.8 kb of the 5'-flanking region of the human NaPi-IIb gene was sequenced and confirmed to be a functional promoter. These experiments resulted in the identification of an EGF-responsive element in the hNaPi-IIb proximal promoter. In

addition, it is determined that the hNaPi-IIb gene promoter had no typical TATA box and a cluster of transcriptional factor binding motifs, including motifs for the nuclear factor 1 (NF1) protein family, was found within 100 bp of the proximal promoter region. The NF1 protein interaction with the basal promoter region is critical for activating the hNaPi-IIb gene. Our work has thus identified the hNaPi-IIb gene as a new target gene directly regulated by the NF1 protein in the intestine. Therefore, EGF can decrease the intestinal Na/Pi absorption at least partially by inhibiting the NaPi-IIb mRNA expression.

3.4 Development

The intestine undergoes dramatic structural and functional changes after birth, such as increasing dry mass and absorptive surface area and changing of membrane permeability and fluidity (Feng et al., 2008). In addition to these nonspecific changes, the absorptive capability per cell (per mg) and the expression of transporters also alters with aging. The intestinal Na-Pi uptake activity and the Na-Pi-IIb protein and mRNA levels are all the highest during the suckling period, and the Na-Pi-IIb protein is not fully glycosylated until weaning. Because the intestine matures very rapidly during the suckling or weaning transition, it is not surprising that suckling animals have a higher capacity to absorb nutrients than older animals. The rapid decline in Na-Pi uptake activity and Na-Pi-IIb expression between the suckling and weaning periods suggests that the Na-Pi transport system matures early in postnatal life. In Western blot analyses from mice at different ages, the molecular mass of Na-Pi-IIb in suckling mice is lower (~88 kDa) than that of the protein in older animals (~110 kDa). The PNGase F Treatment decreased the molecular mass of both Na-Pi-IIb-specific bands (~88 and ~110 kDa) to the predicted size (~78 kDa), whereas the Endo H treatment had no apparent effect. Therefore, our findings strongly suggest that the type IIb Na-Pi cotransporter is an N-linked glycoprotein containing complex oligosaccharides and that partial glycosylation occurs during the suckling or weaning transition.

3.5 Segment

Because of the unique morphological characteristics of the intestine, the distribution of transporters is different along the intestinal axis from proximal to distal segments and from the crypt to villus. Along the mouse small intestine, the Na-Pi cotransporter NaPi-IIb is inhomogenously expressed. The highest abundance of NaPi-IIb protein was found in the ileum, whereas the content of the NaPi-IIb protein was minimal or not detectable in the duodenum and jejunum. On the basis of Na-Pi-transport studies, in mouse small intestine, the transcellular Pi reabsorption occurs to a significant amount in the ileum. Whether such segmental distribution of NaPi-IIb and Na or Pi cotransport is unique for the mouse small intestine or may also be present in other species remains to be analyzed. On the basis of transepithelial Pi flux measurements and uptake studies with isolated brush border membranes (e.g., of rabbits and rats), it has been reported the small intestinal absorption of Pi occurs mostly in the duodenum and at the beginning of the jejunum. There is ample evidence that in these small intestinal segments, the transcellular transport of Pi is initiated by a sodium-dependent transport step through the apical membrane of the enterocytes. In our study, the expression of NaPi-IIb and its correlation with Na/Pi cotransport activity were assessed in the small intestine of the mouse. Interestingly, the highest amount of NaPi-IIb protein was detected in the ileum, whereas in the jejunum, the content of the NaPi-IIb protein was minimal. In agreement, the highest rate of NaPi-IIb cotransport was observed in BBMV isolated from the ileum, whereas in BBMV, isolated from the jejunum, the Na-dependent transport of Pi was not measurable. Although the apical NaPi-llb was detectable by immunofluorescence in the duodenum, NaPi-IIb could not be detected on immunoblots. Also, no Na-dependent Pi uptake could be measured in BBMV isolated from the duodenum. Thus, the described localization of NaPi-llb suggests that in mouse small intestine, the transcellular and Na-dependent absorption of Pi occurs, which is to a large extent in the ileum and minimal in the jejunum and duodenum. Interestingly, it has been shown that in rat small intestine, when considering the different transit times, Pi is absorbed to an equal extent (~1/3 of ingested Pi) in the ileum and in the duodenum (Hilfiker et al., 1998). However, the rat small intestinal localization of the NaPi-llb cotransporter remains to be determined. Finally, studies of the regional profile of phosphate absorption in humans indicate that the proximal small intestine has a greater capacity for absorption of this anion than the distal small intestine (Caverzasio et al., 1987). Thus, according to our present findings, the profile of phosphate absorption (and its likely regulation) along the rat small intestine is much closer to that in humans than in the mouse. The rat is therefore a more appropriate animal model for us to study the processes and regulatory mechanisms influencing phosphate handling along the human small intestine.

3.6 Metabolic acidosis

Metabolic acidosis is associated with several changes in metabolism aiming to restore acid–base homeostasis. Pi plays a key role in the compensation of metabolic acidosis, both as a buffer in blood and as a titratable acid in urine. Gafter et al. (1986) demonstrated that metabolic acidosis led to an increase in the intestinal Na-dependent Pi uptake due to an increased abundance of NaPi-IIb protein in the BBM of the small intestine. This adaptability occurs in the ileum, as shown by immunohistochemistry, and does not alter the segmental distribution of NaPi-IIb protein

localization. Furthermore, the increase in NaPi-IIb protein abundance is not associated with an increase in NaPi-IIb mRNA abundance in the small intestine, which is evident from real-time PCR. In summary, these results demonstrate a strong stimulation of Na-dependent Pi uptake in the BBM of the small intestine of acidotic animals and an increase of NaPi-IIb protein abundance. The increased Pi uptake and delivery may support the compensation of metabolic acidosis and be part of a concerted regulation of mechanisms aiming to restore normal acid–base balance.

4 Conclusions

This article summarizes recent advances on the regulation of the type II sodium-dependent Pi cotransporter (NaPi-IIb) expression, including different mechanisms of transcriptional and posttranscriptional regulation. 1, 25(OH)2 vitamin D3 and dietary Pi deprivation may stimulate the intestinal Na/Pi absorption and NaPi-IIb gene expression via nontransciptional mechanism, whereas the epidermal growth factor (EGF) and glucocorticoids can inhibit the intestinal sodium-dependent Pi (Na/Pi) absorption and NaPi-IIb gene expression via transcriptional mechanism. The estrogen and metabolic acidosis that stimulate the intestinal Na/Pi absorption and NaPi-IIb gene expression via transcriptional mechanism may share the different response elements in the promoter with EGF and glucocorticoids. NaPi-IIb is also regulated by development and shows different segment distribution along the small intestine. These regulators play very important roles in the body phosphate homeostasis.

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