EXPERIMENTAL STUDY

Recovery of renal tubule phosphate reabsorption despite reduced levels of sodium–phosphate transporter

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Abstract

Background: The acute effect of parathyroid hormone (PTH) on phosphate transport has been reported to be mediated by rapid downregulation of sodium–phosphate transporter (NaPi-IIa) protein, but the association was observed with pharmacological doses of PTH.

Objective: To explore the effects of physiological doses of PTH on NaPi-IIa protein and its relationship to phosphate transport.

Methods: Acute clearance studies were performed in parathyroidectomized rats given a bolus i.v. physiological dose (1 µg) of bovine PTH(1–34) and NaPi-IIa protein concentrations were examined at different time intervals.

Results: Fractional excretion of phosphate increased from 0.031 ± 0.006 (mean $\pm$ S.E.) to 0.238 ± 0.059 ($P < 0.01$ compared with baseline and compared with controls) at 40 min and returned to control values by 120 min. Urinary cAMP concentrations were increased at 20 min only. Superficial cortex brush-border membrane (BBM) NaPi-IIa protein was decreased from baseline at both 40 and 120 min ($P < 0.01$) and did not recover at 240 min ($P < 0.01$ compared with baseline and compared with controls).

Conclusion: These results confirm that PTH, even in physiological dosage, causes a rapid decrease in BBM NaPi-IIa, but subsequent recovery of phosphate reabsorption is poorly correlated with BBM concentrations of NaPi-IIa protein. This suggests that transport mechanisms other than NaPi-IIa are important in renal phosphate reabsorption.

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Introduction

Renal proximal tubular epithelial cells express a brush border associated type IIa sodium–phosphate cotransporter (NaPi-IIa). This has been found to be the major mediator of renal phosphate reabsorption (1). Mice deficient in the *NaPi-IIa* gene exhibit 20–30% cotransporter activity as compared with wild-type animals (2). Rapid downregulation of NaPi-IIa occurs during treatment with parathyroid hormone (PTH) as a result of cellular internalization of the NaPi-IIa protein from the apical membrane to endocytosomal vesicles (3). Subsequent degradation of the protein occurs in lysosomes (4). The latter is dependent on microtubule integrity (5). PTH-induced NaPi-IIa endocytosis can be observed within minutes (6), whereas lysosomal degradation of NaPi-IIa may take 3–4 h (7). It should also be noted that pharmacological doses of PTH were used in the *in vivo* experiments referred to here (1, 4–6). Rapid increase (2 h) in

renal brush-border membrane (BBM) NaPi-IIa after dietary restriction of phosphate appears to occur independently of *de novo* protein synthesis and is also dependent on microtubule integrity (8). Full recovery of sodium–phosphate transport after exposure of OK cells to PTH takes 5–7 h (9). This can be blocked by cycloheximide, but not by actinomycin D, suggesting the need for post-translational protein synthesis. However, the *in vivo* phosphaturic effects of physiological doses of PTH are transient and, to our knowledge, BBM concentrations of NaPi-IIa protein after cessation of PTH treatment *in vivo* have not been studied.

We therefore studied the effect of bolus injection of a physiological dose of PTH in parathyroidectomized rats undergoing acute clearance experiments and examined the changes in superficial renal cortex BBM NaPi-IIa protein. We observed that the suppressed rate of tubular phosphate reabsorption returned to normal despite continued reduced BBM NaPi-IIa protein content.

Methods

Animal models

Lightly anaesthetized, parathyroidectomized, white male Sabra rats, weighing 200–240 g and receiving Purina chow (0.67% phosphorus, 0.97% calcium) and with tap water available *ad libitum* underwent acute clearance experiments after catheterization of femoral vessels and the urinary bladder. Rats' kidneys were removed rapidly at the end of the experiments for preparation of superficial cortex BBMs. Parathyroidectomy (PTX) was performed by electric cauterization with the animal under light ether anaesthesia, 2 days before the studies.

Clearance studies

Clearance studies were performed between 0800 h and 1400 h with the animal under intraperitoneal pentobarbitone sodium (40 mg/kg body weight) anaesthesia. In brief, animals were infused i.v. with 0.18% sodium chloride and 4.3% dextrose in water at a rate of 1.5 ml/100 g body weight. After a 60-min equilibration period, a 20-min baseline urine collection was made. A single i.v. bolus of 1 µg bovine PTH(1–34) (Sigma Chemical Co.) was given to each rat. To prevent binding of the PTH to glassware (10), the PTH was first added to 0.25 ml PTX rat serum. Control animals received vehicle only. Subsequently, urine samples were collected under ice cooling, at 20-min intervals. Blood samples were taken after the initial baseline urine collection, when volume was replaced with 0.9% sodium chloride solution, and at the end of the experiment.

Sample analysis

Blood and urine samples were assayed spectrophotometrically for creatinine, calcium and phosphorus using a computer-directed analyser (Cobras Mira, Roche). Urinary cAMP was measured in duplicate by the protein-binding assay of Gilman (11), using a RIA [³H]cAMP assay kit (Amersham).

Isolation of BBMs

Kidneys were rapidly removed and slices were cut from the superficial cortex at 4 °C, homogenized in buffer consisting of 300 mmol/l DL mannitol, 5 mmol/l EGTA, 16 mmol/l Hepes and Tris pH 7.5 containing protease inhibitor cocktail tablets (Boehringer Mannheim GmbH). BBMs were precipitated from this homogenate by double Mg²⁺ precipitation and differential centrifugation as described previously (12). The final pellet was resuspended in the same buffer as above. Protein concentration of the BBM preparation was determined by an automated pyrogallol red colori-

metric method (Cobras Mira) and equal amounts of protein (60 µg) were added to each lane of the polyacrylamide gels.

SDS-PAGE immunoblotting

Aliquots of BBM were denatured 1:1 with sample buffer containing 4% SDS, 20% glycerol, 1% β-mercaptoethanol and 125 mmol/l Tris-HCl, pH 6.8. Sixty micrograms BBM protein per lane were separated on 10% polyacrylamide gels and electrotransferred to nitrocellulose paper. Protein loading equality between the lanes was confirmed before chemiluminescence examination by staining with Ponceau S stain. After blockade with 5% fat-free milk powder, western blots were performed with antiserum against the C-terminal amino acid sequence of NaPi-IIa at a dilution of 1:5000 (1, 13). Secondary antibody was goat anti-rabbit IgG at a dilution of 1:10 000. Antibody binding was visualized using enhanced chemiluminescence; densitometry was performed in a Phosphor Imager.

Statistics

Results are presented as means ± s.e. Analysis of variance was performed for statistical evaluation between different groups. Results between individual groups were compared by a non-paired Student's *t*-test with a modified level of significance according to the Bonferroni method (14).

Results

Effects of PTH on serum chemistries

Laboratory data of the PTX rats are shown in Table 1. There were no significant differences in serum creatinine, calcium or phosphate between the PTH-treated PTX animals and control PTX animals, both at baseline and when the animals were killed. Body weight and creatinine clearance were insignificantly greater in control animals.

Effects of PTH on fractional excretion of phosphate

Fractional excretion of phosphate is shown in Fig. 1. This was maximal at 40 min in PTH-treated animals and thereafter decreased rapidly, albeit remaining significantly greater than in control animals up to 100 min. It should be noted that the rapid decrease in fractional excretion of phosphate after 40 min indicates recovery of renal phosphate reabsorption. This recovery was almost complete at 120 min, and was also evident at 180 and 240 min.

Table 1 Laboratory data.

	Weight (g)	Serum creatinine ($\mu\text{mol/l}$)	Serum calcium (mEq/l)	Serum phosphate (mmol/l)	Creatinine clearance (ml/min)
PTX rats receiving PTH					
Baseline ($n = 15$)	225 $\pm$ 8.3	37.7 $\pm$ 0.58	3.28 $\pm$ 0.14	3.55 $\pm$ 0.10	1.06 $\pm$ 0.09
At harvesting					
40 min ($n = 3$)	240 $\pm$ 20.8	36.1 $\pm$ 2.3	3.57 $\pm$ 0.39	3.67 $\pm$ 0.26	1.07 $\pm$ 0.19
120 min ($n = 8$)	236 $\pm$ 20.8	37.4 $\pm$ 2.74	3.33 $\pm$ 0.15	3.56 $\pm$ 0.09	1.02 $\pm$ 0.06
240 min ($n = 4$)	222 $\pm$ 2.5	40.1 $\pm$ 2.4	3.07 $\pm$ 0.09	3.50 $\pm$ 0.15	0.96 $\pm$ 0.05
Control PTX rats					
Baseline ($n = 9$)	245 $\pm$ 7.4	39.1 $\pm$ 1.6	3.52 $\pm$ 0.07	3.58 $\pm$ 0.13	1.26 $\pm$ 0.12
At harvesting					
120 min ($n = 4$)	235 $\pm$ 5.5	38.5 $\pm$ 2.4	3.31 $\pm$ 0.16	3.15 $\pm$ 0.09	1.32 $\pm$ 0.10

Effects of PTH on urinary cAMP

Figure 2 shows that urinary cAMP excretion peaked at 20 min after PTH injection and had returned to baseline at 40 min.

Effects of PTH on BBM NaPi-IIa protein abundance

Despite the recovery in tubular reabsorption of phosphate, it can be seen in Fig. 3 that BBM NaPi-IIa protein concentrations, which were significantly reduced at 40 min, had not recovered at 120 or at 240 min after the PTH injection, despite full recovery of renal tubular phosphate reabsorption as seen in Fig. 1. Figure 4 shows representative gels from the experiments, showing reduced NaPi-IIa protein at both 40 min and 120 min after the injection of PTH (Fig. 4A), and that the decrease in NaPi-IIa protein was still present at 240 min after PTH injection (Fig. 4B).

Discussion

Tubular epithelial phosphate transport is currently believed to be regulated mainly by the type IIa

sodium–phosphate transporter. Renal expression of NaPi-IIa mRNA and NaPi-IIa protein content in epithelial cell BBMs are markedly influenced by dietary phosphate intake (15). Furthermore, the phosphaturic influence of PTH has been shown to be modulated by cellular internalization of the NaPi-IIa protein from the BBMs, followed by lysosomal degradation (1, 3–8).

We and others have recently noted that BBM expression of NaPi-IIa is not always well correlated with changes in phosphate transport (16–18). It was suggested that the action of PTH may involve changes in NaPi-IIa activity or in the activity or availability of other transporters that influence phosphate transport. In the present study, tubular phosphate reabsorption was acutely inhibited by bolus injection of PTH, which caused an acute and significant decrease in BBM NaPi-IIa protein content. However, the tubular reabsorption of phosphate recovered within 2 h at a time when BBM NaPi-IIa protein content remained acutely reduced. Thus, while confirming that PTH acutely decreases BBM NaPi-IIa protein content even when given in a physiological dose, the findings of our study suggest that the BBM NaPi-IIa protein concentrations are poorly correlated with the subsequent recovery of tubular phosphate transport after an acute bolus injection of PTH. The explanation for this finding may be that the BBM may be contaminated

Figure 1 Fractional excretion of phosphorus (FE-Pi) in PTX rats at baseline (0 min) and after bolus i.v. injection of PTH (1 μg). Control rats received vehicle only.

Figure 2 Urinary cAMP (UcAMP) excretion in rats treated as in Fig. 1.

Figure 3 Superficial cortex BBM NaPi-IIa protein concentrations (arbitrary densitometry units) in rats treated as in Fig. 1. Numbers in parentheses are number of rats.

Figure 4 Representative gels showing NaPi-IIa protein at both 40 and 120 min after the injection of PTH (A), and the decrease in NaPi-IIa still present at 240 min after PTH injection (B). Numbers in parentheses are number of rats.

with vesicles in the endocytosis or exocytosis pathway, that the activity of the NaPi-IIa transporter remaining in the BBM is increased in this situation, that there may be increased renal tubular phosphate reabsorption at tubular sites other than the proximal tubule, or that other sodium–phosphate cotransport proteins that are important for phosphate reabsorption may be activated.

Regarding the first possibility, although we can not rule out for certain that the BBM may be contaminated with vesicles in the endocytosis or exocytosis pathway, western blot studies with isolated BBM have not shown this to be the case.

Regarding the possibility that other sodium–phosphate cotransport proteins that are important for phosphate reabsorption may be activated, in addition to the predominant type IIa sodium–phosphate cotransporter, type IIc, type I and type III sodium–phosphate cotransporters are also expressed in the kidney. In quantitative terms, of the total renal sodium–phosphate cotransporter mRNAs, type I accounts for 15%, type II for 84% and type III for 0.5% (19). NaPi-Ia is expressed in BBMs of both the proximal and the distal tubules. NaPi-Ib is expressed in brain and in erythrocytes. NaPi-Ia mediates sodium–phosphate transport when expressed in *Xenopus laevis* oocytes and is PTH-insensitive. It also operates as a chloride channel and mediates the transport of organic anions (20, 21). Type III cotransporters are cell-surface retroviral receptors, Glvr and ram (Pit 1 and Pit 2). They display widespread renal and extrarenal expression. Type III mRNA is expressed along the entire nephron. It has been proposed that they mediate basolateral phosphate uptake to support cellular energy metabolism. In this regard, they may be viewed as fulfilling a housekeeping function (22). NaPi-IIc, a second renal NaPi-II-related isoform, has been reported in weaning rats and is expressed much less in adult animals (23). It has a high affinity for phosphate, is regulated by dietary phosphate and functions as a major sodium–phosphate cotransporter in rapidly growing weaning rats. Its role is reduced in adult animals, in which NaPi-IIa is the major regulated sodium–phosphate cotransporter. NaPi-IIc exhibits homology with NaPi-IIa and localizes with it exclusively in the proximal tubular BBM. NaPi-Iib is expressed in small intestine, in lung, in prostate, in pancreas and in salivary glands, but not in the mammalian kidney (24). Hybrid depletion studies suggested that NaPi-IIc accounts for 30% of sodium–phosphate cotransporters in the kidneys of phosphate-deprived adult mice (21). NaPi-IIc protein is maximally upregulated in NaPi-IIa knockout mice and therefore is a candidate for residual sodium–phosphate cotransport in BBMs of NaPi-IIa knockout mice. Its protein abundance is 2.7-fold greater in NaPi-IIa knockout mice than in wild-type littermates (21, 22).

Our present observation of recovery of renal phosphate reabsorption despite reduced NaPi-IIa protein concentrations remains unexplained. Further studies are required to explore the possibility that upregulation of other sodium–phosphate cotransporters, including NaPi-IIc, as discussed above and as has been reported in NaPi-IIa knockout mice, could account for the present findings.

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