Magnesium stimulates renal phosphate reabsorption

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Magnesium (Mg2+) is the second most abundant intracellular cation in vertebrates. It interacts with calcium (Ca2+) and phosphate (P) homeostasis in different ways. Mg2+ activates like Ca2+ the Ca2+-sensing receptor (CaSR) of the parathyroid glands (2). Activation of the CaSR results in decreased parathyroid hormone (PTH) secretion. Although Mg2+ is less potent than Ca2+ in stimulating the CaSR, genetic diseases caused by mutations in the CaSR gene suggest that CaSR serves as an Mg2+ sensor in vivo. Individuals with autosomal-dominant hypoparathyroidism (ADH) due to activating mutations of the CaSR show, besides hypercalcicuric hypocacemia, tendencies toward hypomagnesemia and hyperphosphatemia (10). Heterozygous inactivating mutations of the CaSR cause familial hypocalciuric hypercalcemia (FHH), which is characterized by hypercalcicuric hypercalcemia often associated with hypomagnesuria and hypercalciuria (26). Affected individuals may display hypophosphatemia due to decreased renal Pi reabsorption (16). Homozygous or compound heterozygous inactivating mutations of the CaSR result in neonatal severe hyperparathyroidism (NSHPT), which is generally lethal unless a parathyroidectomy is performed (26).

High serum Mg2+ levels may have a suppressive role in PTH secretion in humans (5). In peritoneal dialysis patients, serum Mg2+ concentration is inversely correlated with PTH levels (23, 33). Taken together, these observations point to an influence of Mg2+ on PTH secretion in vivo.

Thus the aim of this study was to elucidate the effect of Mg2+ in renal P1 handling. Overall P1 homeostasis is mainly regulated by intestinal absorption and renal excretion of P1. Approximately 80% of the filtered P1 is reabsorbed along the proximal tubule. Transport of P1 across the proximal tubular cell membrane is mainly initiated by the sodium-dependent P1 cotransport system (NaPi-IIa and NaPi-IIc). Slightly increased serum P1 levels in normocalcemic or hypercalcemic patients are generally due to autocrine or paracrine stimulation of the CaSR by extracellular P1 (19). Mg2+ also stimulates renal phosphate reabsorption. Under a high-Mg2+ diet, NaPi-IIc protein expression was increased in Sprague-Dawley rats (7). In this study, the effect of Mg2+ on renal P1 reabsorption was studied in rats fed a normal (0.2%) or high-Mg2+ (2.5%) diet for 4 wk. Mg2+ increased renal Pi reabsorption by enhancing protein expression of NaPi-IIa and NaPi-IIc. Slightly increased serum P1 levels were measured under a high-Mg2+ diet. In a second set of experiments, renal P1 reabsorption and NaPi-IIa and NaPi-IIc expression were analyzed in parathyroidectomized (PTX) rats fed a normal (0.2%) or high-Mg2+ (2.5%) diet for 4 wk. Mg2+ increased renal P1 reabsorption by enhancing protein expression of NaPi-IIa and NaPi-IIc. Slightly decreased serum P1 levels were measured under a high-Mg2+ diet. Thus the aim of this study was to elucidate the effect of Mg2+ in renal P1 handling. Overall P1 homeostasis is mainly regulated by intestinal absorption and renal excretion of P1. Approximately 80% of the filtered P1 is reabsorbed along the proximal tubule. Transport of P1 across the proximal tubular cell membrane is mainly initiated by the sodium-dependent P1 cotransport system (NaPi-IIa and NaPi-IIc). Slightly increased serum P1 levels in normocalcemic or hypercalcemic patients are generally due to autocrine or paracrine stimulation of the CaSR by extracellular P1 (19). Mg2+ also stimulates renal phosphate reabsorption. Under a high-Mg2+ diet, NaPi-IIc protein expression was increased in Sprague-Dawley rats (7). In this study, the effect of Mg2+ on renal P1 reabsorption was studied in rats fed a normal (0.2%) or high-Mg2+ (2.5%) diet for 4 wk. Mg2+ increased renal Pi reabsorption by enhancing protein expression of NaPi-IIa and NaPi-IIc. Slightly increased serum P1 levels were measured under a high-Mg2+ diet. In a second set of experiments, renal P1 reabsorption and NaPi-IIa and NaPi-IIc expression were analyzed in parathyroidectomized (PTX) rats fed a normal (0.2%) or high-Mg2+ (2.5%) diet for 4 wk. Mg2+ increased renal Pi reabsorption by enhancing protein expression of NaPi-IIa and NaPi-IIc. Slightly increased serum P1 levels were measured under a high-Mg2+ diet. In the kidney, about 80% of the filtered P1 is reabsorbed along the proximal tubule. Changes in renal P1 reabsorption are associated with modulation of the sodium-dependent P1 cotransporter type IIa (NaPi-IIa) and type IIc (NaPi-IIc) protein abundance in the brush-border membrane (BBM) of proximal tubule cells. NaPi-IIa is mainly regulated by dietary P1 intake and parathyroid hormone (PTH). The purpose of the present study was to elucidate the effect of altered diet in magnesium (Mg2+) intake on renal P1 handling. Urinary P1 excretion and renal expression of NaPi-IIa and NaPi-IIc were analyzed in rats fed a normal (0.2%) or high-Mg2+ (2.5%) diet. A high-Mg2+-diet resulted in decreased renal P1 excretion and increased protein expression of NaPi-IIa and NaPi-IIc. Serum FGF-23 (fibroblast growth factor 23) levels were unchanged under a high-Mg2+ diet. Serum PTH levels were slightly decreased under a high-Mg2+ diet. To examine whether the observed changes in renal P1 reabsorption are PTH dependent, expression of NaPi-IIa and NaPi-IIc was also analyzed in parathyroidectomized (PTX) rats fed a normal or high-Mg2+ diet. In PTX rats, Mg2+ had no significant effect on renal P1 reabsorption or NaPi-IIa protein expression. Mg2+ increased NaPi-IIc protein expression in PTX rats. This experiment shows for the first time on the molecular level how Mg2+ stimulates renal P1 reabsorption. Under a high-Mg2+ diet, NaPi-IIa expression is dependent on PTH levels, whereas NaPi-IIc expression seems to be independent of PTH levels.

MATERIALS AND METHODS

Animal studies. The experiments were performed with male Sprague-Dawley rats (300–350 g). Animals were purchased from Charles River Laboratories: 14 PTX rats and 14 control rats. All animal studies were performed according to the APS Guiding Principles in the Care and Use of Laboratory Animals and were approved by the local institutional office (Landesamt für Arbeitsschutz, Gesundheits- und technische Sicherheit Berlin; Number 0001/06). Four different groups (7 animals each) were investigated: group 1 (control), nonoperated rats that received normal drinking water and standard

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Table 1. Serum and urine analysis in normal and PTX rats under a normal diet and high-magnesium diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
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<th>PTX</th>
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<tr>
<td></td>
<td>−Mg²⁺</td>
<td>+Mg²⁺</td>
<td>−Mg²⁺</td>
<td>+Mg²⁺</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/l</td>
<td>141.1 ± 3.7</td>
<td>141.7 ± 1.3</td>
<td>143.0 ± 0.6</td>
<td>143.9 ± 1.9</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion, μmol/day</td>
<td>1,958.1 ± 2,185</td>
<td>1,521.6 ± 477.8</td>
<td>1,751.5 ± 551.6</td>
<td>1,327.6 ± 397.2*</td>
</tr>
<tr>
<td>Serum K⁺, mmol/l</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Urinary K⁺ excretion, μmol/day</td>
<td>3,523.3 ± 390.2</td>
<td>3,657.3 ± 752.3</td>
<td>3,193.0 ± 807.2</td>
<td>3,208.6 ± 589.9</td>
</tr>
<tr>
<td>AP, U/l</td>
<td>121.4 ± 21.3</td>
<td>125.3 ± 18.5</td>
<td>141.0 ± 24.6</td>
<td>125.3 ± 12.1</td>
</tr>
<tr>
<td>Urinary pH</td>
<td>8.5 [8.5; 8.5]</td>
<td>8.5 [8.5; 8.5]</td>
<td>8.5 [8.5; 8.5]</td>
<td>8.5 [8.5; 8.5]</td>
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Values are means ± SD; n = 7 animals/group. Urinary pH is presented as median [range]. PTX, parathyreoidectomized; −Mg²⁺, normal diet; +Mg²⁺, high-magnesium diet; AP, alkaline phosphatase. *P < 0.05 vs. control group under a normal diet.

Fig. 1. Serum Ca²⁺, Mg²⁺, P, parathyroid hormone (PTH) and FGF-23 concentration and urinary Ca²⁺, Mg²⁺, and P, excretion in control and parathyroidectomized (PTX) rats under a normal (−Mg²⁺) and high-magnesium diet (+Mg²⁺); n = 7 animals/group. Values are means ± SD. *P < 0.05 vs. control group under a normal diet. #P < 0.05 vs. PTX group under a normal diet.
pelleted chow (R/M-F extradrate from ssniff, 0.2% Mg\(^{2+}\)) ad libitum; group 2 (control + Mg\(^{2+}\)), nonoperated rats that received a high-Mg\(^{2+}\) diet (drinking water supplemented with 2.5% Mg\(^{2+}\)-sulfate and standard pelleted chow ad libitum); group 3 (PTX), PTX rats that received normal drinking water and standard pelleted chow (0.2% Mg\(^{2+}\)) ad libitum; and group 4 (PTX + Mg\(^{2+}\)), PTX rats that received a high-Mg\(^{2+}\) diet (drinking water supplemented with 2.5% Mg\(^{2+}\)-sulfate and standard pelleted chow ad libitum). The feeding period lasted for 28 days. At the end, rats were housed in metabolic cages for 24-h urine collection. Animals were anesthetized with ketamine and xylazine injected intraperitoneally. Blood samples were taken. Kidneys were perfused with ice-cold PBS solution via the abdominal aorta and removed.

**Analytic procedures.** Serum concentrations of Mg\(^{2+}\), Ca\(^{2+}\), P, Na\(^{+}\), K\(^{+}\), and alkaline phosphatase were determined. Twenty-four-hour urine samples were analyzed for pH, Mg\(^{2+}\), Ca\(^{2+}\), P, Na\(^{+}\), and K\(^{+}\). Intact PTH serum levels were measured by an immunoradiometric assay specific for rat intact PTH (Immunotopics, San Clemente, CA). Primers used for amplification of NaPi-IIa were forward 5'-cggatagttcatgggccatccat-3', reverse 5'-caaaactcgtggagctcactgct-3'. Expression of NaPi-IIa and GAPDH, as an endogenous control, were determined by quantitative real-time PCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

**Immunoblotting.** Kidneys were homogenized in iced lysis buffer (20 mmol/l Tris, pH 7.4, 5 mmol/l MgCl\(_2\), 1 mmol/l EDTA, 0.3 mmol/l EGTA) enriched with protease inhibitor cocktail complete mini (Boehringer Mannheim) according to the manufacturer's instructions. Insoluble material was removed by centrifugation at 250 g for 5 min at 4°C. For obtaining the membrane protein fraction, the supernatant was then centrifuged at 43,000 g for 30 min at 4°C. The pellet was resuspended in iced lysis buffer. Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL).

Aliquots of 25 μg (for NaPi-IIa and NaPi-Ic) and 50 μg protein (for β-actin) were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (NEN Life Science Products, Boston, MA). After the blots were blocked for 2 h in 5% milk powder in PBS and overnight in 5% bovine serum albumin in PBS containing 0.1% Tween 20, they were incubated with the primary antibodies (mouse anti-β-actin, Sigma-Aldrich, St. Louis, MO; 1:100,000, rabbit anti-NaPi-IIa, 1:1,000, and rabbit anti-NaPi-Ic, 1:1,000) for 3 h at room temperature (RT). Secondary peroxidase-conjugated goat anti-rabbit and goat anti-mouse IgG antibodies and the chemiluminescence detection system Lumi-LightPlus (Roche) were used to detect bound antibodies. Densitometric comparison was performed on the same immunoblot.

**Immunohistochemistry.** Kidneys were fixed in a solution containing 4% formalin and 10% sucrose in PBS for 1 h at RT. After fixation, kidneys were stored in 20% sucrose in PBS for 1 h at RT and in 30% sucrose in PBS overnight at 4°C. Kidneys were then frozen in liquid isopentane cooled with liquid nitrogen. For immunofluorescence staining, 5-μm-thick sections were pretreated with PBS containing 0.5% Triton X-100 in PBS for 15 min. After being blocked with 5% goat serum for 1 h, sections were incubated with the primary antibody NaPi-IIa (1:1,000) in 5% goat serum for 3 h at 37°C. After being washed with 5% goat serum, sections were incubated with the secondary antibody Alexa Fluor 594 (Invitrogen) diluted 1:500 in 5% goat serum for 45 min at RT. Fluorescence images were obtained using a confocal scanning microscope (LSM 510, Carl Zeiss, Jena, Germany). For semiquantitative determination of protein abundance, images were analyzed with the internal software, resulting in quantification of the protein levels as the mean of integrated optical density (IOD) in arbitrary units.

**Statistical analysis.** Data are expressed as means ± SD. All data were tested for significance using ANOVA followed by an unpaired Student’s t-test. \(P\) values <0.05 were considered significant.

**RESULTS**

Metabolic changes induced by magnesium. The metabolic data obtained under a normal diet (0.2% Mg\(^{2+}\)) and high-Mg\(^{2+}\) diet (2.5% Mg\(^{2+}\)) over 4 wk in control and PTX rats are shown in Table 1 and Fig. 1. A high-Mg\(^{2+}\) diet had no effect on serum Na\(^{+}\) concentration in control and PTX rats. Urinary Na\(^{+}\) excretion in PTX rats under a high-Mg\(^{2+}\) diet was significantly reduced compared with control rats under a normal diet. A high-Mg\(^{2+}\) diet had no effect on serum or urinary K\(^{+}\) levels. Serum alkaline phosphatase levels were not altered under a high-Mg\(^{2+}\) diet in control and PTX rats. Urinary pH was unchanged in the different groups. Total Mg\(^{2+}\) serum concentration and urine Mg\(^{2+}\) excretion were significantly elevated in control and PTX rats under a high-Mg\(^{2+}\) diet. Urinary Ca\(^{2+}\) excretion was also significantly elevated in control and PTX rats under a high-Mg\(^{2+}\) diet. Total serum Ca\(^{2+}\) levels in PTX rats were significantly lower than in control rats. Under a high-Mg\(^{2+}\) diet, total serum Ca\(^{2+}\) levels increased in PTX rats, whereas total serum Ca\(^{2+}\) levels in control rats were unchanged under a high-Mg\(^{2+}\) diet. PTX rats had significantly lower urinary Pi excretion compared with control rats. Serum P\(_{i}\) concentration was significantly higher in PTX rats than in control rats. Under a high-Mg\(^{2+}\) diet, urinary \(P_{i}\) excretion was significantly reduced in control rats. A high-Mg\(^{2+}\) diet had no effect on serum P\(_{i}\) concentration in PTX rats.
rats, urinary Pi excretion was not significantly decreased under a high-Mg\(^{2+}\) diet. Serum PTH levels in PTX rats were significantly lower compared with control rats. A high-Mg\(^{2+}\) diet tended to result in lower PTH levels in control rats, whereas low PTH levels in PTX rats were not affected by magnesium. Serum FGF-23 (fibroblast growth factor 23) levels were significantly lower in PTX rats compared with control rats. A high-Mg\(^{2+}\) diet tended to result in lower PTH levels in control rats, whereas low PTH levels in PTX rats were not affected by magnesium. A high-Mg\(^{2+}\) diet had no effect on serum FGF-23 levels in both groups.

Expression of NaPi-IIa in normal vs. PTX rats under a normal diet. NaPi-IIa protein expression was analyzed by Western blotting (Fig. 2) and immunohistochemistry (see Figs. 3–5). NaPi-IIa protein expression in the whole kidney membrane protein fraction was significantly higher in PTX rats compared with control rats (Fig. 2). In control rats under a normal diet, proximal tubules of midcortical and superficial nephrons showed weak NaPi-IIa staining (Fig. 3) as described previously (6). While NaPi-IIa protein staining in the BBM was moderate, there was also staining in the cytoplasm of the proximal tubule cells, most likely reflecting NaPi-IIa protein residing in the Golgi apparatus (Fig. 4). Lowered PTH levels increased NaPi-IIa staining especially in proximal tubules of midcortical and superficial nephrons (Fig. 3). Under these conditions, NaPi-IIa protein was exclusively located in the BBM; no signal in the cytoplasm was detectable (Fig. 4). Differences in NaPi-IIa staining in juxtamedullary nephrons were less conspicuous (Fig. 5). NaPi-IIa mRNA expression was analyzed by quantitative real-time PCR in whole kidney and was unchanged in PTX rats compared with control group (Table 2).

Fig. 3. Effect of a high-magnesium diet (+Mg\(^{2+}\)) in control and PTX rats on NaPi-IIa abundance in the outer renal cortex. Arrows indicate the renal capsule. A: representative immunohistochemical image of NaPi-IIa staining. B: semiquantitative determination of NaPi-IIa staining intensity by computerized analysis. Data are depicted as ratio to control group under a normal diet; n = 7 animals/group. Values are means ± SD. *P < 0.05 vs. control group under a normal diet.
Expression of NaPi-IIa in normal vs. PTX rats under a high-Mg\(^2+\)/H\(_{11001}\) diet. A high-Mg\(^2+\)/H\(_{11001}\) diet significantly increased NaPi-IIa protein expression in whole kidney membrane protein fraction in control rats (Fig. 2). Under a high-Mg\(^2+\) diet, NaPi-IIa protein-related staining was particularly increased in midcortical and superficial nephrons (Fig. 3). NaPi-IIa was primarily located in the BBM of proximal tubule cells; no intracellular signal was detectable (Fig. 4). Changes in NaPi-IIa staining in juxtamedullary nephrons were more moderate (Fig. 5). In PTX rats, Mg\(^2+\) had no effect on total NaPi-IIa protein expression analyzed by Western blotting and immunohistochemistry (Figs. 2–5). There was no change in NaPi-IIa staining intensity in the proximal tubule of rat renal cortex (Figs. 3–5). NaPi-IIa mRNA expression was unchanged under a high-Mg\(^2+\) diet in control and PTX rats (Table 2).

Expression of NaPi-IIc in normal vs. PTX rats under a normal and high-Mg\(^2+\) diet. NaPi-IIc protein expression was analyzed by Western blotting (Fig. 6). NaPi-IIc protein expression in the whole kidney membrane protein fraction was significantly higher in PTX rats compared with control rats. A high-Mg\(^2+\) diet significantly increased NaPi-IIc protein expression in whole kidney membrane protein fractions in control rats and PTX rats (Fig. 6).

DISCUSSION

In the present study, the effect of Mg\(^2+\) on renal P\(_i\) handling in rats was investigated. A high-Mg\(^2+\) diet increased renal P\(_i\) reabsorption by enhancing protein expression of NaPi-IIa and NaPi-IIc transporters. Increased NaPi-IIa protein expression in rats during high Mg\(^2+\) intake was dependent on serum PTH levels. NaPi-IIc protein expression increased independently from serum PTH levels, suggesting an alternative mechanism of action.

The effect of either Mg\(^2+\) supplementation or depletion on Ca\(^2+\)-Pi homeostasis in rats (7, 8, 12, 13, 25, 28, 30) and other species (19, 31) has been investigated before. In these studies, changes observed in Ca\(^2+\)-Pi homeostasis were not congruent. One reason for these differences might be variances in dietary Mg\(^2+\) content and duration of the feeding period. In the present study, urinary Ca\(^2+\) excretion increased significantly with no change in total serum Ca\(^2+\) concentration under a high-Mg\(^2+\) diet. Increased urinary Ca\(^2+\) excretion might be due to competitive paracellular Ca\(^2+\)/Mg\(^2+\) reabsorption in the thick ascending limb of Henle mediated by claudin 16 (11), stimulation of the CaSR in the thick ascending limb of Henle, inhibition of PTH release (2), or competition for the TRPV5 calcium channel (4).

Urinary P\(_i\) excretion decreased significantly under a high-Mg\(^2+\) diet with no change in serum P\(_i\) levels in our study. This observation is in accordance with the study of Ginn et al. (8): under Mg\(^2+\) deprivation, urinary P\(_i\) excretion increased significantly without affecting serum P\(_i\) levels. Fiore et al. (7) also found no change in serum P\(_i\) level under Mg\(^2+\) supplementation; urinary P\(_i\) excretion was not investigated. Massry et al. (19) found elevated serum P\(_i\) levels and decreased fractional P\(_i\) excretion under Mg\(^2+\) supplementation. In the present study,
serum PTH levels seemed to be lower under Mg$^{2+}$/H11001 supplementation, but this was not statistically significant probably due to large variances in the control group. In the above-mentioned studies, the effect of Mg$^{2+}$/H11001 on serum PTH levels varied widely. Riond et al. (28) and Katsumata et al. (13) observed no changes in serum PTH levels under high Mg$^{2+}$/H11001 diet, whereas Fiore et al. (7) found decreased serum PTH levels under a high-Mg$^{2+}$/H11001 diet. Mg$^{2+}$/H11001 deficiency in rats caused suppressed, unchanged, or elevated serum PTH levels (12, 25, 30). Slatopolsky et al. (31) showed that hypermagnesemia in dogs inhibits the renal action of PTH, resulting in less urinary P$_i$/H11001 excretion.

In all these studies, the underlying molecular mechanisms responsible for the observed changes in P$_i$/H11001 metabolism under Mg$^{2+}$/H11001 deprivation or supplementation were not elucidated.

Table 2. mRNA expression of NaPi-IIa in normal and PTX rats under a normal diet and a high-magnesium diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+Mg$^{2+}$/H11001</th>
<th>PTX</th>
<th>+Mg$^{2+}$/H11001</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaPi-IIa, copies in ratio to GAPDH</td>
<td>0.132±0.067</td>
<td>0.045±0.016</td>
<td>0.103±0.079</td>
<td>0.158±0.113</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 animals/group. NaPi-IIa, sodium-dependent P$_i$/H11001 cotransporter type IIa.
Renal Pi reabsorption is regulated by a variety of hormonal and metabolic factors. Insulin and Pi deprivation stimulate renal Pi reabsorption, whereas PTH, FGF-23, and metabolic acidosis reduce Pi reabsorption (1, 17, 21). The rate-limiting step of transcellular Pi reabsorption is the apical entry of Pi in the proximal tubule cell. This is mainly initiated by NaPi-IIa. NaPi-IIa expression is heterogeneously distributed within the renal cortex. Under basal conditions, i.e., normal dietary phosphate intake, NaPi-IIa protein abundance in the BBM is highest in juxtaglomerular nephrons, whereas protein abundance in superficial and midcortical nephrons is low (3). Enhanced renal Pi reabsorption is caused by an increased abundance of NaPi-IIa protein in the BBM especially in superficial and midcortical nephrons of proximal tubules (18, 29). The regulation of renal NaPi-IIc expression has been studied less. Metabolic acidosis increases NaPi-Ic protein expression without changing the abundance via an alternative indirect pathway. The animals in our experiment received Mg2+ -sulfate, which can cause mild metabolic acidosis and acidification of urine (9). Acidosis is known for enhancing Pi excretion (1). In our experiment, no change in urinary pH under Mg2+-supplementation was observed. The phosphatonin FGF-23 also stimulates renal Pi excretion by decreasing NaPi-IIa expression (17). In our experiment, a high-Mg2+-diet had no effect on serum FGF-23 levels. Thus it is unlikely that Mg2+ stimulates NaPi-IIa expression through changes in acid-base balance or changes in FGF-23 secretion.

Mg2+ can activate like Ca2+ but less potently than the CaSR of the parathyroid glands (2). Activation of the CaSR decreases PTH secretion. In our study, Mg2+ seemed to decrease PTH levels, although PTH changes did not reach significance. To investigate whether Mg2+ stimulates PTH reabsorption in a PTH-dependent or -independent manner, a second experiment with PTX rats was performed. Apparently, parathyroidecmy was only partial as PTH was still detectable in serum although at significantly lower levels than in control rats. Serum Pi concentration was elevated, while urinary Pi excretion was reduced compared with control rats as expected. Total serum Ca2+ concentration was decreased in PTX rats. Taken together, these changes in Ca2+-Pi homeostasis indicate an effective reduction of biologically active PTH in the PTX rats (27).

Serum FGF-23 levels were significantly reduced in PTX rats in our study. Correlation between PTH and FGF-23 secretion is not completely clarified. While Kobayashi et al. (15) found elevated FGF-23 levels in subjects with primary hyperparathyroidism, Tebben et al. (32) found no changes in FGF-23 serum levels in subjects with primary hyperparathyroidism compared with healthy subjects. Recent animal studies indicate that 1,25-(OH)2 vitamin D3 stimulates FGF-23 synthesis, while activation of 25-OH vitamin D3 by the 1α-hydroxylase enzyme is under the control of PTH (20). Thus PTH may control FGF-23 levels via 1,25-(OH)2-vitamin D3. Consistently, in our study deprivation of PTH was associated with decreased serum FGF-23 levels.

A high-Mg2+ diet in PTX rats resulted in increased urinary Ca2+ excretion like in control rats. However, total Ca2+ serum concentration was elevated under Mg2+-supplementation in PTX rats. These findings cannot only be explained by competitive paracellular Ca2+/Mg2+ reabsorption in the thick ascending limb of Henle (11) or stimulation of the CaSR in the thick ascending limb of Henle and inhibition of PTH release (2). Alternatively, Bonny et al. (4) recently suggested that high urinary Mg2+ may compete for reabsorption with Ca2+ at the TRPV5 channel in the distal convoluted and connecting tubule. The mechanism responsible for elevating serum Ca2+ levels in this case is not known.

NaPi-IIa protein expression in the whole kidney membrane protein fraction increased after parathyroidecmy in our study (Fig. 2). NaPi-IIa protein abundance, particularly in midcortical and superficial nephrons, was enhanced (Figs. 3 and 4). Changes in expression of NaPi-IIa mRNA were not observed. These findings are in accordance with Kempson et al. (14), who observed increased NaPi-IIa protein expression in the BBM without changes in mRNA expression in PTX rats. Supplementation of Mg2+ after parathyroidecmy had no additional effect on total NaPi-IIa protein expression or distribution of NaPi-IIa protein within the renal cortex (Figs. 2–5). Under a high-Mg2+-diet, urinary excretion of Pi, seemed to be lower in PTX rats. Mg2+ supplementation increased NaPi-IIc expression in PTX rats. This fact could explain the slightly increased Pi reabsorption under a high-Mg2+-diet after parathyroidecmy. This suggests that Mg2+ regulates NaPi-IIc expression independently from PTH levels. Maybe Mg2+ has
an additional effect on renal $P_i$ excretion, besides lowering PTH levels. It could directly stimulate the CaSR of proximal tubule cells or modulate the action of other phosphaturic or antiphosphaturic factors, including PTH (31). These data indicate that the main effect of Mg$^{2+}$ is not directly influencing renal $P_i$ reabsorption. Rather, Mg$^{2+}$ inhibits PTH secretion, which results in increased renal $P_i$ reabsorption by increasing NaPi-IIa protein expression. Therefore, these experiments show for the first time on the molecular level how Mg$^{2+}$ influences renal $P_i$ excretion. Moreover, these data also confirm previous findings that Mg$^{2+}$ influences PTH secretion not only in vitro but also in vivo (2, 5, 10, 16, 23, 26, 33).

GRANTS

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