Intestinal and renal adaptation to a low-P_i diet of type II NaPi cotransporters in vitamin D receptor- and 1αOHase-deficient mice

Paola Capuano,1 Tamara Radanovic,1 Carsten A. Wagner,1 Desa Bacic,1 Shigeaki Kato,2 Yasushi Uchiyama,3 René St.-Arnoud,4 Heini Murer,1 and Jürg Biber1

1Institute of Physiology; University of Zurich, Zurich, Switzerland; 2Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan; 3Fuj Gotemba Research Laboratories, Shizuoka, Japan; and 4Genetic Unit, Shriners Hospital, Montreal, Quebec, Canada

Submitted 8 July 2004; accepted in final form 20 September 2004

Capuano, Paola, Tamara Radanovic, Carsten A. Wagner, Desa Bacic, Shigeaki Kato, Yasushi Uchiyama, René St.-Arnoud, Heini Murer, and Jürg Biber. Intestinal and renal adaptation to a low-P_i diet of type II NaPi cotransporters in vitamin D receptor- and 1αOHase-deficient mice. Am J Physiol Cell Physiol 288: C429–C434, 2005; doi:10.1152/ajpcell.00331.2004.—Intake of a low-phosphate diet stimulates transepithelial transport of P_i in small intestine as well as in renal proximal tubules. In both organs, this is paralleled by a change in the abundance of the apically localized NaPi cotransporters NaPi_Ia (NaPi-Ia) and NaPi type Ib (NaPi-Ib), respectively. Low-P_i diet, via stimulation of the activity of the renal 25-hydroxyvitamin-D_3-1α-hydroxylase (1αOHase), leads to an increase in the level of 1,25-dihydroxy-vitamin-D_3 [1,25(OH)2D]. Regulation of the intestinal absorption of P_i and the abundance of NaPi-IIb by 1,25(OH)2D has been supposed to involve the vitamin D receptor (VDR). In this study, we investigated the adaptation to a low-P_i diet of NaPi-Ib in small intestine as well as NaPi-IIb in kidneys of either VDR- or 1αOHase-deficient mice. In both mouse models, upregulation by a low-P_i diet of the NaPi cotransporters NaPi-Ia and NaPi-Ib was normal, i.e., similar to that observed in the wild types. Also, in small intestines of VDR- and 1αOHase-deficient mice, the same changes in NaPi-Ib mRNA found in wild-type mice were observed. On the basis of the results, we conclude that the regulation of NaPi cotransport in small intestine (via NaPi-Ib) and kidney (via NaPi-IIa) by low dietary intake of P_i cannot be explained by the 1,25(OH)2D-VDR axis.

NaPi_Ib; vitamin D_3

IN SMALL INTESTINE AND RENAL proximal tubules, transepithelial transport of P_i is initiated by members of the type II Na^+ dependent phosphate cotransporter family SLC34 (18), which are localized at the apical membrane of the respective epithelial cells. In adult mice, renal reabsorption of P_i is determined largely by the abundance of NaPi_Ia (NaPi-IIa) in the brush-border membrane vesicles (BBMV) of proximal tubular cells and in small intestine by NaPi_Ib (NaPi-Ib), the only apical NaPi cotransporter known to be involved in the absorption of P_i (14, 19). Thus regulation of intestinal absorption and renal reabsorption of P_i is mainly explained by alterations in the apical abundances of NaPi-IIb and NaPi-IIa, respectively (12, 15, 19, 28). Low dietary intake of P_i is a well-known stimulator of both renal and small intestinal P_i handling (3, 6, 16). In both organs, low-P_i diet provokes an increase of the abundance of both type II NaPi cotransporters. In mouse intestine, the increase in NaPi-IIb protein is also paralleled by an increase in NaPi-IIb mRNA (23; Radanovic T, Wagner CA, Murer H, and Biber J, unpublished data), whereas in proximal tubules of adult mice, an increase in NaPi-IIa mRNA was not observed (17).

Low dietary intake of P_i stimulates the synthesis of 1,25-dihydroxy-vitamin D_3 [1,25(OH)2D] via stimulation of the renal 25-hydroxyvitamin-D_3-1α-hydroxylase (1αOHase; for review, see Ref. 2). In turn, the stimulation of intestinal P_i absorption by a low-P_i diet has been attributed to an increased level of 1,25(OH)2D (3, 6). In agreement with these findings, it was shown that the status of 1,25(OH)2D influences the abundance of the apical NaPi-IIb protein (12, 28). Because low-P_i diet induces an upregulation of NaPi-IIb mRNA (23; Radanovic T, Wagner CA, Murer H, and Biber J, unpublished data), it could be envisaged that the action of 1,25(OH)2D is via a genomic mechanism that involves the vitamin D receptor (VDR) (2). The role of 1,25(OH)2D in the renal handling of P_i is less clear, notably also because of the complexity rooted in an interrelationship with the status of parathyroid hormone (PTH) (8).

In the present study, we investigated the regulation of NaPi-IIb in enterocytes and NaPi-IIa in proximal tubules by a low-P_i diet in VDR- and renal 1αOHase-deficient mice. Our results demonstrate that in both mouse models, NaPi-IIb (protein and mRNA) and NaPi-IIa (protein) are upregulated by a low-P_i diet to a degree similar to that in wild-type animals. On the basis of the presented results, we conclude that in neither intestine nor kidney does the VDR or 1,25(OH)2D play a central role in the adaptive phenomenon induced by a low-P_i diet.

METHODS

Animals. All experiments were performed with male mice aged 10–12 wk. Two different batches of VDR−/− mice (originally described in Ref. 29) were used. As controls, wild-type C57BL/6 mice were purchased from Charles River Laboratories (Sulzfeld, Germany). After weaning, VDR−/− mice were fed a high-Ca^2+ diet (2%) to rescue the abnormalities observed in these animals (16a). (For a description of 1αOHase-deficient mice, see Ref. 7.) As controls, the corresponding heterozygotes were used because it has been demonstrated that 1αOHase heterozygotes do not show any differences from the wild types (7). Before experiments, all animals were maintained for 2 wk on a standard diet (0.8% P_i, 1.2% Ca^2+). Afterward, the animals were fed diets containing either high P_i (1.1%) or low P_i (0.1%) (Kliba; NAFAG, Kaiseraugst, Switzerland) for 5 days. Both

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
diets contained 1.0% Ca\(^{2+}\). During all feeding periods, the animals had free access to normal tap water. Animal care and experimentation were performed according to the regulations of the Veterinary Authority of Zurich (Zurich, Switzerland).

**Determination.** Plasma and urinary concentrations of phosphate and creatinine were determined on the basis of spontaneous urine samples and heparinized serum using commercial kits (Sigma Diagnostics, Munich, Germany, and Wako Chemicals, Reinach, Switzerland) according to the manufacturers' protocols. Total protein was determined using Coomassie blue reagent (Bio-Rad).

**Incubation of kidney slices and immunofluorescence.** Incubation and processing of kidney slices was performed according to a previously described method (1). After a 45-min incubation in either the absence or the presence of 10\(^{-7}\) M bovine 1-34 PTH (Sigma), slices were fixed for 4 h, rinsed with PBS, and immunostained for NaPi-IIa as described elsewhere (1, 5).

**Isolation of membranes. Western blot analysis, and NaPi transport measurements.** Kidney cortex tissue was homogenized, and crude membranes were prepared by performing differential centrifugation. From whole small intestines, BBMV were isolated using a Mg\(^{2+}\)-dependent transport of Pi into isolated small intestine BBMV was determined at 25°C and a final concentration of 0.3 mM Pi in the presence of inwardly directed gradients of either 100 mM NaCl or 100 mM KCl (13).

**Real-time PCR.** Small intestines were rinsed with cold 0.9% NaCl and inverted, and the mucosa from late jejunum up to the early ileum was scraped off. For each preparation, the intestinal mucosa of two or three animals was pooled.

For Western blot analysis, 50 μg of total protein were separated by SDS-PAGE and analyzed for NaPi-IIa, NaPi-IIb, calbindin D9k, and β-actin after transfer onto nitrocellulose membranes according to standard procedures using 5% fat-free milk powder as a blocking reagent (5, 14). The following primary antibodies were used: anti NaPi-IIa and anti NaPi-IIb, as described previously (5, 14), anti-calbindin D9k (SWANT, Bellinzona, Switzerland), and anti β-actin (no. 3516; Sigma). Immunoreactions were detected using the corresponding secondary HRP-conjugated IgG (Amersham) and enhanced chemiluminescence (SuperSignal; Pierce Biotechnology). Quantitative analysis was performed with digital imaging (Diana III; Raytest, Straubenhardt, Germany).

Na\(^{+}\)-dependent transport of Pi into isolated small intestine BBMV was determined at 25°C and a final concentration of 0.3 mM Pi in the presence of inwardly directed gradients of either 100 mM NaCl or 100 mM KCl (13).

For Western blot analysis, 50 μg of total protein were separated by SDS-PAGE and analyzed for NaPi-IIa, NaPi-IIb, calbindin D9k, and β-actin after transfer onto nitrocellulose membranes according to standard procedures using 5% fat-free milk powder as a blocking reagent (5, 14). The following primary antibodies were used: anti NaPi-IIa and anti NaPi-IIb, as described previously (5, 14), anti-calbindin D9k (SWANT, Bellinzona, Switzerland), and anti β-actin (no. 3516; Sigma). Immunoreactions were detected using the corresponding secondary HRP-conjugated IgG (Amersham) and enhanced chemiluminescence (SuperSignal; Pierce Biotechnology). Quantitative analysis was performed with digital imaging (Diana III; Raytest, Straubenhardt, Germany).

Na\(^{+}\)-dependent transport of Pi into isolated small intestine BBMV was determined at 25°C and a final concentration of 0.3 mM Pi in the presence of inwardly directed gradients of either 100 mM NaCl or 100 mM KCl (13).

**Real-time PCR.** Small intestines were rinsed with cold 0.9% NaCl and inverted, and mucosa from late jejunum up to the early ileum was frozen immediately. Total RNA was extracted using the RNAeasy kit (Qiagen). Reverse transcription was performed using random hexamers (TaqMan reverse transcription reagents; Applied Biosystems). The sequences of TaqMan probes (Applied Biosystems) used for the amplification of NaPi-IIb were as follows: 5'-6-FAM TGGTCA-GAGAGAGACAC (BHQ-1)-3' (probe), 5'-CCTGGGACCTGGCT-GAACT-3' (forward primer), and 5'-AATGCAGAGCCTCTC-CCTTT-3' (reverse primer).

The probes to amplify β-actin cDNA were described elsewhere (17). The relative expressions of NaPi-IIb mRNA to the β-actin mRNA were calculated according to the method described in User Bulletin 2 for the ABI Prism 7700 Sequence Detection System (http://docs.appliedbiosystems.com/pebiodocs/04303859.pdf).

For each condition, mucosa of three animals was collected. Three independent rounds comprising reverse transcription and real-time (RT)-PCR were performed. In each round, samples from one animal for each condition were analyzed. Because the values of the relative NaPi-IIb mRNA contents (expressed as the ratio of NaPi-IIb mRNA to β-actin mRNA) varied between the different rounds, the results (see Fig. 6) are expressed as relative upregulation (low- vs. high-Pi diet) of the relative NaPi-IIb mRNA contents.

**Statistical analysis.** Data are presented as means ± SD. Student's t-test was used to test for significance of differences between mice of the same strain fed a high- or a low-Pi diet. P < 0.05 was considered significant.

**RESULTS**

Urinary excretion of phosphate of wild-type animals, VDR-deficient mice, and 1αOHase-deficient mice was determined over a period of several days during which the dietary content of Pi was varied as indicated in Fig. 1. As illustrated, after switching from a normal to a low-Pi diet, urinary excretion was reduced to almost zero and there was no difference between the wild types and both strains of knockout mice. In contrast, while both knockout groups were fed a high-Pi diet, their normalized urinary excretion of Pi was significantly reduced compared with that of the corresponding wild types. Similarly, reduced Pi excretion was observed while mice were fed a normal laboratory chow diet containing 0.8% Pi. No difference was observed with regard to urinary Pi excretion of VDR\(^{-/-}\) and 1αOHase\(^{-/-}\) mice fed a high-Pi diet.

The concentrations of Pi in the serum of animals fed a high- or a low-Pi diet for 5 days are listed in Table 1. After 5 days on a high-Pi diet, serum Pi in the VDR\(^{-/-}\) animals did not differ from that of the wild types, whereas the 1αOHase\(^{-/-}\) animals showed moderate hypophosphatemia, which is in agreement with earlier reports (7, 26). In contrast to data published by others (23), the serum Pi concentration in VDR-deficient mice fed a high-Pi diet was not different from that of the wild-type mice. In all animals, low-Pi diet resulted in a hypophosphatemia that was more pronounced in both knockouts compared with the wild types. After low-Pi diet, serum Pi levels of the VDR\(^{-/-}\) and the 1αOHase\(^{-/-}\) mice did not differ significantly. The values for the fractional excretion of Pi (FE\(_{Pi}\), %) in animals fed a high-Pi diet were not significantly different from each other.
between the wild-type and VDR−/− mice and OHase+/− and OHase−/− mice, respectively (Table 1). FEPi values of all animals fed a low-Pi diet were close to zero.

Because renal excretion of Pi is determined largely by the abundance of the type IIa NaPi cotransporter in the apical membranes of proximal tubular cells (19), the total abundance of NaPi-IIa contained in a crude membrane fraction of renal cortex tissue was analyzed using immunoblotting (Fig. 2). In VDR−/−, 1αOHase−/− mice fed a high-Pi diet, the abundance of NaPi-IIa did not differ significantly. After feeding both wild-type and VDR-deficient animals 1-34 PTH for 45 min. As illustrated, PTH led to downregulation of NaPi-IIa in slices derived from the wild types as well as in slices derived from the VDR−/− mice. Similar observations were made with kidney slices obtained from 1αOHase-deficient mice (data not shown). In addition, the cellular localization of the NaPi-IIa-interacting proteins NHERF-1 and PDZK1 (11) was analyzed using immunofluorescence. The apical localization of those proteins was not disturbed by the deficiency in VDR or 1αOHase (data not shown).

In the small intestine, the type II NaPi cotransporter NaPi-IIb is involved in the absorption of Pi (12, 14). To investigate whether VDR or 1αOHase is required for the upregulation of NaPi-IIb by a low-Pi diet, BBMV were isolated from small intestines of animals fed a high- or a low-Pi diet and analyzed for the abundance of NaPi-IIb by performing Western blot analysis and for NaPi cotransport. As illustrated in Fig. 4, the low-Pi diet provoked an increase in NaPi-IIb abundance in both knockout models that was comparable to the upregulation of NaPi-IIb in BBMV obtained from the corresponding control animals. In VDR−/− as well as 1αOHase−/− animals fed a high-Pi diet, the abundance of NaPi-IIb tended to be less than that of the wild types. The same BBMV preparations were analyzed for calbindin D9k, which was previously described to be involved in the absorption of Pi (12, 14). To investigate the downregulation and cellular localization of NaPi-IIa in the VDR−/− samples was normal; i.e., NaPi-IIa was almost exclusively localized to the apical membrane, and its abundance was highest in the S1 segments. In parallel, kidney slices obtained from animals fed a low-Pi diet were incubated with 1-34 PTH for 45 min. As illustrated, PTH led to downregulation of NaPi-IIa in slices derived from the wild types as well as in slices derived from the VDR−/− mice. Similar observations were made with kidney slices obtained from 1αOHase-deficient mice (data not shown). In addition, the cellular localization of the NaPi-IIa-interacting proteins NHERF-1 and PDZK1 (11) was analyzed using immunofluorescence. The apical localization of those proteins was not disturbed by the deficiency in VDR or 1αOHase (data not shown).

In the small intestine, the type II NaPi cotransporter NaPi-IIb is involved in the absorption of Pi (12, 14). To investigate whether VDR or 1αOHase is required for the upregulation of NaPi-IIb by a low-Pi diet, BBMV were isolated from small intestines of animals fed a high- or a low-Pi diet and analyzed for the abundance of NaPi-IIb by performing Western blot analysis and for NaPi cotransport. As illustrated in Fig. 4, the low-Pi diet provoked an increase in NaPi-IIb abundance in both knockout models that was comparable to the upregulation of NaPi-IIb in BBMV obtained from the corresponding control animals. In VDR−/− as well as 1αOHase−/− animals fed a high-Pi diet, the abundance of NaPi-IIb tended to be less than that of the wild types. The same BBMV preparations were analyzed for calbindin D9k, which was previously described to be regulated by 1,25(OH)2D (2). As expected, upregulation of calbindin D9k by a low-Pi diet was detected in BBMV of wild types but not in BBMV derived from VDR-deficient mice. In agreement with previously published data (7), calbindin D9K was absent in the 1αOHase−/− mice (data not shown). Na+-dependent uptake of Pi was determined with BBMV isolated from wild-type and VDR−/− animals.

In agreement with the data obtained by Western blot analysis, NaPi cotransport into BBMV derived from VDR−/− animals was upregulated by a low-Pi diet (Fig. 5). Similar results were obtained with BBMV isolated from and OHase−/− mice (data not shown).

Because a low-Pi diet leads to an increase in the abundance of NaPi-IIa mRNA in the small intestines of wild-type mice.

Table 1. Urine and serum parameters and fractional excretion of Pi

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>VDR−/−</th>
<th>1αOHase+/−</th>
<th>1αOHase−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+Pi</td>
<td>−Pi</td>
<td>+Pi</td>
<td>−Pi</td>
</tr>
<tr>
<td>Urinary Pi, excretion-to-Pi creatinine ratio*</td>
<td>10.3±1.9</td>
<td>0.32±0.11</td>
<td>5.89±0.88</td>
<td>0.39±0.13</td>
</tr>
<tr>
<td>Serum Pi, mM</td>
<td>1.70±0.14</td>
<td>0.70±0.10</td>
<td>1.89±0.13</td>
<td>0.44±0.11</td>
</tr>
<tr>
<td>FE Pi, %</td>
<td>4.5±1.6</td>
<td>0.11±0.1</td>
<td>5.5±1.5</td>
<td>0.66±0.22</td>
</tr>
<tr>
<td></td>
<td>+Pi</td>
<td>−Pi</td>
<td>+Pi</td>
<td>−Pi</td>
</tr>
<tr>
<td></td>
<td>12.41±2.3</td>
<td>0.47±0.3</td>
<td>6.80±0.71</td>
<td>0.24±0.07</td>
</tr>
</tbody>
</table>

Data represent means ± SD; n = 4. VDR, vitamin D receptor; 1αOHase, 25-hydroxyvitamin-D-1α-hydroxylase. Serum Pi, and fractional excretion index of Pi (FE Pi) were determined in animals that had been fed (after the second normal period shown in Fig. 1) for 5 days with a high- or a low-Pi diet. *Values are taken from Fig. 1 at days 5 and 9, respectively. †P < 0.05, significantly different from OHase+/− (+Pi).

Downloaded from http://ajpcell.physiology.org/ by 10.220.33.1 on June 16, 2017
(23; Radanovic T, Wagner CA, Murer H, and Biber J, unpublished results), the contents of NaPi-IIb mRNA were determined by performing RT-PCR and compared with the contents of the \( \beta \)-actin mRNA. As displayed in Fig. 6, in both VDR\(^{-/-}\) and \( \alpha \)OHase\(^{-/-}\) mice, NaPi-IIb mRNA was similarly upregulated by a low-P\(_i\) diet as observed in wild-type and OHase\(^{-/-}\) mice.

DISCUSSION

Low-P\(_i\) diet represents a classic stimulator of transepithelial transport of P\(_i\) in renal proximal tubules and the small intestine. In both organs, a low-P\(_i\) diet provokes an increase in the abundance of apically located type II NaPi cotransporters: NaPi-IIa and NaPi-IIc in proximal tubules and NaPi-IIb in small intestine (12, 16, 21). The precise mechanisms of regulation are still not entirely clear. One prominent event elicited by a low-P\(_i\) diet is an increase in the activity of the renal mitochondrial \( \alpha \)OHase, which results in an increased level of 1,25(OH)\(_2\)D (reviewed in Ref. 2). Thus part of the restoration of phosphate homeostasis, when impaired by a low-P\(_i\) diet, has been explained by a stimulation of intestinal absorption of P\(_i\) and bone resorption by 1,25(OH)\(_2\)D (3, 6, 22). In fact, it has been demonstrated that the abundance of the NaPi cotransporter NaPi-IIb is altered by changes in vitamin D status (12, 15, 28). Only recently it was reported that a low-P\(_i\) diet leads to an increase in the abundance of NaPi-IIb mRNA as well (23; Radanovic T, Wagner CA, Murer H, and Biber J, unpublished results). Therefore, the latter finding suggests that an increased level of 1,25(OH)\(_2\)D would result in increased transcription of NaPi-IIb mRNA, possibly involving the VDR (2). In the kidney, the role of 1,25(OH)\(_2\)D in \( P_i \) reabsorption, and more specifically its effect on the abundance of NaPi-IIa cotransporter in proximal tubules, is less clear. Conflicting results have been reported indicating that a possible action of 1,25(OH)\(_2\)D on the NaPi-IIa cotransporter is dose dependent and depends on alterations in the level of PTH (8, 19). As demonstrated recently, a genomic effect of low-P\(_i\) diet on the upregulation of NaPi-IIa seems unlikely, because in cortical as well as juxtamedullary proximal tubular S1 and S3 segments of adult mice, the abundance of NaPi-IIa mRNA was not altered by a low-P\(_i\) diet (17).

In the present study, we used VDR\(^{-/-}\) and \( \alpha \)OHase\(^{-/-}\) mice to investigate the roles of the VDR and elevated levels of
1,25(OH)2D in the adaptation of NaPi-IIa in kidney and NaPi-IIb in small intestine to a low-Pi diet. Our results demonstrate that in VDR−/− and 1αOHase−/− mice, the adaptation to a low-Pi diet of the NaPi-IIa and NaPi-IIb proteins and NaPi-IIb mRNA was normal, i.e., comparable to that of the respective wild-type animals. Similar results were reported recently for VDR-deficient mice (23).

As indicated by the determinations of urinary excretion of Pi, by Western blot analysis of NaPi-IIa, VDR−/− and 1αOHase−/− mice adapted to a low-Pi diet similarly to the wild types. On the other hand, under high-Pi diet conditions, urinary excretion of Pi was less in both knockout models compared with the wild types, and similar values for the fractional excretion of Pi were observed in the knockout mice and the corresponding wild-type mice. Decreased urinary excretion of Pi was not paralleled by detectable changes in the abundance of the NaPi-IIa protein as one would assume to occur because of increased levels of PTH reported for VDR- and 1αOHase-deficient mice (7, 23). In contrast to our findings, slight decreases in NaPi-IIa abundance have been reported in VDR-deficient mice (23), 1,25(OH)2D-deficient rats (25), and 1αOHase-deficient weaning mice (26). Because the regulation of NaPi-IIa by PTH may be impaired by the lack of 1,25(OH)2D (10), kidney slices of adapted mice were incubated with PTH. These experiments demonstrated that the signaling machinery involved in the downregulation of NaPi-IIa by PTH was intact and did not depend on the presence of the VDR or on an increased level of 1,25(OH)2D. Therefore, the lack of a decreased abundance of NaPi-IIa in the knockout mice may be explained by the fact that, in our study, mice were fed a high-Pi diet, a condition that leads to complete down-regulation of NaPi-IIa (see Fig. 3). Under such conditions, additional downregulation of NaPi-IIa by PTH may be too small to be detected. Of note, reported reductions in NaPi-IIa content (23, 25, 26) were observed in animals fed a normal Pi diet containing 0.6–0.8% Pi.

Another type II NaPi cotransporter, NaPi-IIc, was recently described and localized to the renal proximal tubules (21). The observed reduced urinary Pi excretion of mice fed a high-Pi diet may therefore be explained by a change in the content of the NaPi-IIc cotransporter. However, NaPi-IIc was related to growth and is of very low abundance in adult mice. Furthermore, the abundance of NaPi-IIc was reported not to be altered in VDR-deficient mice (23).

In the aggregate, our results suggest that the expression of the NaPi-IIa protein in the renal proximal tubules of mice fed a high-Pi diet is not affected by the status of 1,25(OH)2D or by the VDR. However, in both knockout mice, less urinary excretion of Pi was observed, despite similar values for the fractional excretion of Pi in the knockout mice compared with the corresponding controls. As mentioned above, this finding can be explained by small, undetectable changes in NaPi-IIa abundance in mice fed a high-Pi diet. Furthermore, our results demonstrate that the upregulation of NaPi-IIa due to a low-Pi diet does not depend on an increase in the level of 1,25(OH)2D via stimulation of renal 1αOHase and does not require the VDR. In accordance, it was reported that to blunt the stimulation of 1αOHase after hypophysectomy, the adaptation of NaPi cotransport by feeding a low-Pi diet to rats was normal (27).

Numerous studies have shown that intestinal absorption of Pi is regulated by 1,25(OH)2D (3, 6). Furthermore, it has been demonstrated that upregulation of intestinal Pi absorption can be explained by an alteration of the apical abundance of the NaPi-IIb protein (12, 15, 28). In adult mice, an increase in the NaPi-IIb protein is paralleled by an increase in NaPi-IIb mRNA (23; Radanovic T, Wagner CA, Murer H, and Biber J, unpublished results). The latter finding suggests that upregulation of NaPi-IIb occurs via a genomic mechanism possibly involving the VDR. On the other hand, it has been postulated that 1,25(OH)2D may act on the basis of rapid, nongenomic mechanisms (9). In this context, a protein of chicken enterocytes that belongs to the superfamily of glucose-regulated and redox-sensitive proteins was recently described as a 1,25(OH)2D-binding protein (20). However, in mouse small intestine, a change in the abundance of the NaPi-IIb protein was not observed 3 h after the onset of a low-Pi diet (12), indicating that a rapid nongenomic mechanism may not be involved in the regulation of NaPi-IIb in mouse small intestine.

As shown in the present study, upregulation of the NaPi-IIb protein by a low-Pi diet in VDR−/− mice (see also Ref. 23) and

![Fig. 6. Real-time PCR of NaPi-IIb mRNA. Total RNA was isolated from late jejunum and early ileum of mice fed a high or a low-Pi diet for 5 days. Bars represent relative (−fold) upregulation by a low-Pi diet of the relative contents of NaPi-IIb mRNA (ratios of NaPi-IIb mRNA to β-actin mRNA). Each bar represents the result obtained from 1 animal.](http://ajpcell.physiology.org/)
1αOHase-deficient mice is comparable to that in wild-type mice. Also, upregulation of NaPi-IIb mRNA by feeding a low-Pi diet to the knockout mice strains was comparable to that observed in the wild-type mice. Therefore, we conclude that the upregulation of the NaPi-IIb protein by feeding mice a low-Pi diet involves a genomic mechanism that does not include the 1,25(OH)2-D3-VDR axis; i.e., it does not require the VDR and is not dependent on an increase in the level of 1,25(OH)2-D via stimulation of the renal 1αOHase.

ACKNOWLEDGMENTS

We thank C. Madjdpour for performing the initial RT-PCR experiments.

GRANTS

We thank the Swiss National Science Foundation for financial support under Grant 31-61438 (to J. Biber).

REFERENCES