Inorganic phosphate homeostasis in sodium-dependent phosphate cotransporter Npt2b+/− mice

Akiko Ohi,1* Etsuyo Hanabusa,1* Otoya Ueda,2 Hiroko Segawa,1 Naoshi Horiba,2 Ichiro Kaneko,1 Shoji Kuwahara,1 Tomo Mukai,1 Shohei Sasaki,1 Rieko Tominaga,1 Junya Furutani,1 Fumito Aranami,1 Shuichi Ohtomo,2 Yumiko Oikawa,1 Yousuke Kawase,3 Naoko A. Wada,4 Takanori Tachibe,4 Mami Kakefuda,1 Hiromi Tateishi,3 Kaoru Matsumoto,3 Sawako Tatsumi,1 Shinsuke Kido,1 Naoshi Fukushima,2 Kou-ichi Jishage,2,3 and Ken-ichi Miyamoto1

1Department of Molecular Nutrition Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima City; and 2Chugai Pharmaceutical Company, Limited, and 3Chugai Research Institute for Medical Science, Incorporated, Tokyo, Japan

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INTESTINAL ABSORPTION OF INORGANIC phosphate (Pi) has been characterized in several mammalian and avian species (5, 28, 31, 32). Studies conducted with isolated intestinal brush-border membrane vesicles (BBMVs) have demonstrated that the trans-

epithelial uptake of Pi occurs primarily in the proximal and distal small intestine secondary to passive diffusion across the intestinal brush border and Na+-dependent, carrier-mediated uptake (5, 29, 30). Three types of sodium-dependent Pi cotransporters have been identified (5). The type II sodium phosphate transporter family is important for epithelial Pi transport (5, 6, 10, 29). Type IIa (Npt2a), type IIc (Npt2c), and type III (PiT-2) sodium-phosphate transporters are expressed in the apical membrane of renal proximal tubular cells and contribute to renal Pi reabsorption (5, 6, 10, 29). Intestinal absorption of Pi is mediated primarily via the type IIb sodium-phosphate transporter Npt2b (29, 40, 43, 44). 1,25-Dihydroxyvitamin D3 and dietary Pi depletion are thought to be the most important physiological stimuli of intestinal Pi absorption and act by increasing the abundance of Npt2b protein (11, 16, 27, 42).

In chronic renal failure, Pi retention leads to secondary hyperparathyroidism and uremic bone disease and progression to end-stage renal disease (13, 14, 25). The current therapy for hyperphosphatemia in patients with chronic renal failure consists of dietary Pi restriction in combination with administration of Pi binders, but each therapy has practical problems (8, 24). Dietary restriction of Pi is limited by poor patient compliance (20, 21). Therefore, other therapeutic approaches to reduce serum Pi are needed. In addition, in animals with chronic renal failure (CRF), phosphate transport (via the Npt2b protein) across the small intestine is not reduced (23). Therefore, Npt2b is a primary target for the development of inhibitors of hyperphosphatemia (23). We previously reported that Npt2b inhibitor significantly decreases intestinal Pi absorption in normal animals (26).

Recently, Sabbagh et al. (35) demonstrated that in Npt2b conditional knockout (KO) mice, intestinal Npt2b plays a major role in Pi absorption and homeostasis. They also reported that Npt2b is largely responsible for intestinal Pi absorption and contributes to the maintenance of systemic Pi homeostasis (35). The roles of Npt2b in CRF mice, however, remain unknown. In the present study, we characterized Pi homeostasis in Npt2b−/− mice. The present study demonstrated that Npt2b−/− mice have significantly reduced intestinal Na+-dependent Pi co-transport activity in the BBMVs. In addition, plasma Pi concentrations in the Npt2b+/− mice with adenine-induced renal failure were significantly decreased compared with Npt2b+/+ mice.
MATERIALS AND METHODS

Construction of the Npt2b gene-targeting vector. The bacterial artificial chromosome (BAC) clone carrying a genomic DNA containing the full-length mouse Npt2b gene was purchased from Invitrogen (Carlsbad, CA). To construct a targeting vector for Npt2b gene disruption, the BAC clone was modified using the Red/ET system (Quick and Easy BAC Modification kit, GeneBridges) (3, 33). First, a PCR product containing the neomycin-resistant gene cassette (pgk-gb2-NeoR, GeneBridges) was used to replace exons 2–4 of Npt2b on the BAC clone. A NotI site was then created at ~1.5 kb upstream from the pgk-gb2-NeoR insertion site for linearization of the targeting construct before electroporation into embryonic stem (ES) cells (35).

Generation of Npt2b gene KO mice. The targeting vector was introduced by electroporation (200 V, 500 μF) into mouse ES cells (TX-MES-01, Thromb-X N.V.), which were established from 129SvEvTacfBr. The ES cells were selected in a medium containing G418. G418-resistant ES cell clones were screened by PCR using the primers Npt2b 74991 (5′ = TGGACCACATCTCAGCCATAC-3′) and pgk-antisense (5′ = GCTGTCCATCTGCACGAGAC-3′) to select homologous recombinants. Homologous recombinant ES cell clones were injected into C57BL/6J (B6) mouse (CLEA Japan) blastocysts to produce chimera mice. Chimera mice were bred with B6 females to generate offspring. Germline transmission of the mutation was determined by PCR analysis using tail genomic DNA of the offspring. The positions of the PCR primers are shown in Fig. 1A. To detect the null allele, the PCR product was amplified by the Npt2b 74991 and pgk-antisense primer set. After an initial denaturation at 95°C for 1 min, 35 cycles (95°C for 30 s, 66°C for 30 s, and 72°C for 90 s) were run using TaKaRa LA Taq (TaKaRa, Japan). To detect the wild-type allele, the PCR product was amplified by the Npt2b 74991 and Npt2b P4 (5′-GACCATTTGATCCCAGTGTCC-3′) primer set. After an initial denaturation at 94°C for 2 min, 30 cycles (94°C for 30 s, 68°C for 2 min) were run using TaKaRa LA Taq. PCR products of null and wild-type alleles were detected at 1.4 and 1.5 kb, respectively. Typical results of PCR analysis are shown in Fig. 1B.

Genotypes were determined by analyzing DNA obtained from each mouse at ~10 days after birth. Genomic DNA was extracted from tail clippings and amplified by PCR using primers specific for the Npt2b+/− exon 2–4 or for the neomycin-resistant gene. Novel primers with the sequences of 5′-GATGTGTGTATCTGAGCTTGTC-3′ and 5′-CGAGGTCGACGGTATCGATA-3′ were prepared for Npt2b and 5′-TTGCTCCTCTCGGCTTTCTGG-3′ and 5′-CGATACCGTAGAACGAGG-3′ were prepared for detection of the neomycin resistance gene (Fig. 1A).

Analysis of embryos. At 9.5–11.5 days postcoitum (dpc; plug date, 0.5 dpc), the embryos were dissected free of the decidua and yolk sac,
the amnion was removed, and the embryos were then photographed under stereomicroscopic observation. Tissue from each embryo was used for genotyping. Genotyping was performed by PCR according to the above-described method for tail DNA.

Animals and diet. Mice (Npt2b+/+ and Npt2b−/−) were weaned at 3 wk of age and provided free access to water and standard mice chow (1.12% Ca and 0.9% P; Oriental, Osaka, Japan). The Npt2b−/− and Npt2b+/+ mouse phenotypes were analyzed at 4, 9, and 20 wk of age. For the mouse CRF model, adenine-induced nephritis was produced in male mice at 9 wk of age by orally administering adenine dissolved in carboxymethyl cellulose (see Fig. 8 for the administration schedule). To induce adenine-induced nephritis, 7-wk-old male mice were administered adenine (50–100 mg/kg body wt) dissolved in carboxymethyl cellulose three times orally (36).

Mice were maintained under pathogen-free conditions and handled in accordance with the Guidelines for Animal Experimentation of Tokushima University School of Medicine or with the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical.

Preparation of BBMVs and transport assay. The mouse intestine was divided into two segments. The proximal intestine refers to the duodenum and the proximal part of the jejunum. The distal intestine refers to the late jejunum and ileum (16). BBMVs were prepared from intestine using the Ca2+-dependent Pi transport activity (activity rate in the presence of Na+/H+ exchange) as described previously (17, 38). Levels of leucine aminopeptidase, Na+/K+-ATPase, and cytochrome c oxidase were measured to assess membrane purity. There were no significant differences between the intestinal BBMVs of Npt2b+/+ and Npt2b+/− mice regardless of whether they were or were not treated with adenine. Uptake of 32P into BBMVs was measured by the rapid transport rate into the intestinal BBMV was determined at 60 s and at 60 min (equilibrium value) at 25°C with an inward gradient of 100 mM NaCl2 or 100 mM KCl and 0.1 mM KH2PO4 (pH 7.5). The 32P uptake was divided into two segments. The proximal intestine refers to the late jejunum and ileum (16). BBMVs were prepared from intestine using the Ca2+-dependent Pi transport activity (activity rate in the presence of Na+/H+ exchange) as described previously (17, 38). Levels of leucine aminopeptidase, Na+/K+-ATPase, and cytochrome c oxidase were measured to assess membrane purity. There were no significant differences between the intestinal BBMVs of Npt2b+/+ and Npt2b+/− mice regardless of whether they were or were not treated with adenine. Uptake of 32P into BBMVs was measured by the rapid filtration technique as described previously (17, 38). The P/Na+/H+ transport rate into the intestinal BBMV was determined at 60 s and at 60 min (equilibrium value) at 25°C with an inward gradient of 100 mM NaCl2 or 100 mM KCl and 0.1 mM KH2PO4 (pH 7.5). The Na+-dependent P, transport activity (activity rate in the presence of Na+ or in the absence of Na+) is shown. All measurements were performed in triplicate.

Immunoblotting. Protein samples were heated at 95°C for 5 min in sample buffer in the presence of 2-mercaptoethanol and subjected to SDS-PAGE. The separated proteins were transferred by electrophoresis to Immobilon-P, polyvinylidene difluoride (Millipore, Billerica, MA) and then treated with diluted antibodies as follows: affinity-purified anti-Npt2a (1:8,000), anti-Npt2c (1:1,000), and anti-Npt2b antibody (1:2,000; Alpha Diagnostics, San Antonio, TX), and anti-PiT-1 antibody (1:1,000; Santa Cruz Biotechnology) (37, 38). Mouse anti-actin monoclonal antibody (Chemicon, Temecula, CA) was used as an internal control. Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG was utilized as the secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA), and signals were detected using Immobilon Western (Millipore).

Concentrations of P, Ca, 1,25(OH)2D3, fibroblast growth factor 23, and creatinine. Concentrations of plasma and urinary inorganic P, and Ca were determined by the Phospha-C test and Calcium-E tests (Wako, Osaka, Japan), respectively. Concentrations of plasma 1,25(OH)2D3 were determined using a 1,25-(OH)2-D vitamin D ELISA Kit (Immundiagnostik, Bensheim, Germany). Concentrations of plasma fibroblast growth factor 23 (FGF23) proteins were determined using the FGF-23 ELISA kit (Kainos Laboratories, Tokyo, Japan). Concentrations of creatinine were determined using LabAssay Creatinine (Wako). Metabolic cages were used to collect the 24-h urine samples.

Statistical analysis. Data are expressed as means ± SE. Differences among multiple groups were analyzed by ANOVA. The significance of differences between two experimental groups was established by ANOVA followed by Student’s t-test. A P value of <0.05 was considered significant.

RESULTS

Generation of Npt2b gene KO mice. Heterozygous mice were obtained from three clones, 1C4, 1H1, and 4H5, which were indistinguishable from their wild-type littermates with respect to size, body weight, behavior, and reproductive ability. To obtain Npt2b−/− mice, heterozygous male and female mice were intercrossed. Of the offspring delivered, no Npt2b−/− mice were produced; there were 222 heterozygous and 105 wild-type offspring (data not shown). This ratio of genotypes was compatible with the laws of Mendelian inheritance if the Npt2b−/− mice were embryonically lethal. At 9.5–11.5 dpc, 50 embryos were recovered from intercrosses of Npt2b+/+ mice. Approximately 20% of the recovered embryos had abnormal morphology or were resorbed. Most embryos with abnormal morphology were determined to be wild-type or heterozygotes. These data indicate that the Npt2b−/− mice are embryonically lethal.

Body weight and growth and expression of Npt2b in Npt2b+/− mice. Npt2b+/− mice were viable, had normal growth, and were without apparent abnormalities (Fig. 2A). Body weight development in heterozygous male and female mice fed the standard diet is illustrated in Fig. 2B. Until 20 wk of age, both genotypes showed a progressive body weight gain with no differences in body weight (Fig. 2B).

Intestinal Npt2b levels were determined in Npt2b+/− and Npt2b+/+ mice fed the standard mice chow (Fig. 2, C and D). In the proximal intestine and distal intestine, Npt2b mRNA levels were significantly decreased by ~50% in Npt2b+/− mice at 4 wk of age (Fig. 2C). In the proximal intestine of Npt2b+/− mice, Npt2b protein amounts were markedly lower compared with those in the distal intestine as described previously (34). The Npt2b protein levels in the proximal intestine were not significantly different between Npt2b+/− mice and Npt2b+/− mice at 4 wk of age. The amounts of Npt2b protein in the distal intestine were decreased in Npt2b+/− mice compared with those in Npt2b+/+ mice at 4 wk of age (Fig. 2D). Similar results were observed in mice at 20 wk of age (percentage of Npt2b protein expression in distal intestine of Npt2b+/− vs. Npt2b+/+; 100 ± 4.55: 58.96 ± 1.64%).

The levels of PiT-1 mRNA in the proximal intestine were significantly increased in Npt2b+/− mice at 20 wk of age, but not in Npt2b+/− mice at 4 wk of age (Supplemental Fig. 1-I and II; all supplemental material for this article is accessible on the journal website). However, the PiT-1 protein expression levels were not significantly changed in both the proximal and distal intestine of Npt2b+/− mice compared with those of Npt2b+/+ mice (Supplemental Fig. 1-III).

Plasma P, Ca, vitamin D, FGF23, and urinary P, and Ca excretion in Npt2b+/− mice. Plasma P, Ca, 1,25(OH)2D3, and FGF23 levels in Npt2b+/− and Npt2b+/+ mice fed the standard mice chow are shown in Fig. 3. At 4 wk of age, plasma P, and Ca levels were significantly decreased in the Npt2b+/− mice compared with the Npt2b+/+ mice (Fig. 3A, a and b). Plasma 1,25(OH)2D3 levels were significantly increased in the Npt2b+/− mice compared with the Npt2b+/+ mice (Fig. 3A, c). Furthermore, plasma FGF23 levels were significantly decreased in the Npt2b+/− mice compared with the Npt2b+/+ mice (Fig. 3Ad). The Npt2b+/− mice also had significantly lower urinary P, excretion compared with Npt2b+/+ mice (Fig.
persisting for 5 min (data not shown). Na/H11001 matched Npt2b
Be (Fig. 3).

Ab transport activity in the distal intestine affects plasma Pi levels
4, and 20 wk of age, renal Npt2a and Npt2c mRNA and protein
levels were significantly increased in Npt2b+/− mice compared with
Npt2b+/- mice at 4 wk of age, but not in the mice at 20 wk of
age (Fig. 5, Ac, Ad, Bc, and Bd). The levels of renal PiT-1/2
mRNA were not significantly changed in Npt2b+/− mice at 4
and 20 wk of age (Supplemental Fig. 2-I and II).

Expression of 25-hydroxyvitamin D-1α-hydroxylase and 25-
hydroxyvitamin D-24-hydroxylase mRNA in the kidney of
Npt2b+/− mice. To further investigate P i homeostasis, we
analyzed vitamin D metabolism in the kidney of Npt2b+/− and
Npt2b+/- mice at 4 and 20 wk of age (Fig. 6). At 4 wk of age, the
25-hydroxyvitamin D-1α-hydroxylase (1α-OHase) mRNA
levels were significantly increased in Npt2b+/− mice compared
with Npt2b+/- mice (Fig. 6a). In contrast, 25-hydroxyvita-
mim D-24-hydroxylase (24OHase) mRNA levels were
significantly decreased in Npt2b+/− mice compared with Npt2b+/-
mice at 4 wk of age (Fig. 6b). At 20 wk of age, there were no
differences in 1α-OHase and 24OHase mRNA levels between
Npt2b+/− and Npt2b+/- mice (Fig. 6b).

Adenine-induced renal failure model. To determine whether
reduced Npt2b protein levels affect plasma P i levels in renal
failure, we used an experimental mouse model with adenine-
induced renal failure with hyperphosphatemia (Fig. 7). On
day 4, body weight was significantly decreased in adenine-
Npt2b+/− and Npt2b+/- mice compared with control mice
(Fig. 7b). Plasma creatinine and P i levels were significantly
increased in adenine-Npt2b+/− mice compared with
adenine-Npt2b+/- mice and adenine-Npt2b+/- mice compared with non-

3 Ae). In contrast, urinary Ca excretion levels were significantly
increased in the Npt2b+/− compared with Npt2b+/- mice
(Fig. 3Af).

At 20 wk of age, plasma P i, Ca, 1,25(OH)2D3, and FGF23
levels were not different between Npt2b+/− and Npt2b+/-
mice (Fig. 3B, a–d). Urinary P i excretion was significantly
decreased in 20-wk-old Npt2b+/− mice compared with age-
matched Npt2b+/- mice as described in the mice at 4 wk of age
(Fig. 3Be). Urinary Ca excretion was not different between
Npt2b+/− and Npt2b+/- mice (Fig. 3Bf).

Intestinal Na+/H11001-dependent P i transport activity in Npt2b+/−
mice. At 4 and 20 wk of age, intestinal BBMV Na+/H11001-
dependent P i transport activity was assessed in Npt2b+/− and
Npt2b+/- mice fed the standard mice chow (Fig. 4). Sodium-dependent
P i uptake was linear for up to 60 s, with a slow increase
persisting for 5 min (data not shown). Na+/H11001-dependent P i
transport activity in the proximal intestine tended to be reduced in the
Npt2b+/− mice compared with Npt2b+/- mice at both 4
and 20 wk of age, but not significantly (Fig. 4, Aa and Ba). In
contrast, Na+/H11001-dependent P i transport activity was significantly
decreased in the distal intestine of Npt2b+/− mice compared
with that of the Npt2b+/- mice at both 4 and 20 wk of age (Fig.
4, Ab and Bb). These data suggest that Na+/H11001-dependent P i
transport activity in the distal intestine affects plasma P i levels
and urinary P i excretion.

Expression of renal Npt2a and Npt2c in Npt2b+/− mice. At
4 and 20 wk of age, renal Npt2a and Npt2c mRNA and protein
levels were determined in Npt2b+/− and Npt2b+/- mice fed a
normal P i diet (Fig. 5). Npt2a and Npt2c mRNA levels were
not different between Npt2b+/− and Npt2b+/- mice (Fig. 5,
Aa, Ab, Ba, and Bb). Npt2a and Npt2c protein levels were

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Fig. 2. Characterization of Npt2b+/− mice. A: phenotype of 9-wk-old mice Npt2b+/+ and Npt2b+/− mice. Each point represents the mean ± SD body weight derived from 6–15 mice. C: Npt2b mRNA levels in the intestine of Npt2b+/+ and Npt2b+/− mice, as assessed by quantitative PCR. GAPDH was used as an internal control. Male 4-wk-old mice (n = 4–5). Values are means ± SD. *P < 0.05 vs. Npt2b+/+ mice. D: Western blot analysis of brush-border membrane vesicles (BBMVs) isolated from the proximal and distal intestine from Npt2b+/+ and Npt2b+/− mice. Each lane was loaded with 15 μg BBMV. Actin was used as an internal control.
treated mice, respectively (Fig. 7E). The levels of Npt2b protein in the distal intestine were not significantly different between adenine-treated and nontreated mice (Fig. 7F). The levels of Npt2b protein were significantly lower in adenine-treated Npt2b/H11001/H11002 mice than in adenine-treated Npt2b/H11001/H11001 mice (Fig. 7F). The levels of NaPi transport activity were significantly reduced in Npt2b/H11001/H11002 mice compared with Npt2b/H11001/H11001 mice, as described in Fig. 4. There were no differences, however,

Fig. 3. Plasma and urine parameters in Npt2b/+/+ and Npt2b+/-/- mice. A: male 4-wk-old mice (n = 5–6). B: male 20-wk-old mice (n = 6–8). a, Plasma Pi; b, plasma Ca; c, plasma 1,25(OH)2D3; d, plasma FGF23; e, urinary Pi/urinary creatinine (Cr); f, urinary Ca/urinary Cr. Metabolic cages were used for 24-h urine collection from Npt2b+/-/- and Npt2b+/-/- mice. Values are means ± SD. *P < 0.05 vs. Npt2b+/-/- mice.

Fig. 4. Intestinal BBMV Na+/Pi-dependent Pi transport activity in Npt2b+/-/- and Npt2b+/-/- mice. A: male 4-wk-old mice (n = 5–7). B: male 20-wk-old mice (n = 5 to 7). a, Na+/Pi-dependent Pi transport activity in the proximal intestine; b, Na+/Pi-dependent Pi transport activity in the distal intestine. Values are means ± SD. *P < 0.05 vs. Npt2b+/-/- mice.
between control and adenine-treated mice (nontreated vs. adenine-treated; in Npt2b/H11001/H11001 mice, 0.62 ± 0.04 vs. 0.59 ± 0.12 nmol, in Npt2b/H11002/H11002 mice, 0.29 ± 0.13 vs. 0.32 ± 0.18 nmol).

**DISCUSSION**

To study the role of Npt2b in vivo, we generated Npt2b gene KO mice. There were no Npt2b/H11002/H11002 offspring at birth derived from intercrossing Npt2b/H11001/H11002 mice, indicating that Npt2b/H11002/H11002 mice are embryonically lethal. Recently, Shibasaki et al. (39) reported that Npt2b/H11002/H11002 embryos die by 10.5 dpc during early embryogenesis, which could be due to Pi deficiency at the parietal endoderm prior to the establishment of the placenta, where embryonic and maternal circulation are in close contact. Embryonic lethality of Npt2b/H11002/H11002 mice strongly suggests that Npt2b has an essential role in supplying Pi to embryos by active transport during early embryogenesis. In contrast, the heterozygotes (Npt2b/H11002/H11002) were viable. Therefore, we characterized the phenotypes of heterozygous mice with regard to Pi homeostasis.

In the present study, we investigated the amounts of Npt2b protein in Npt2b/H11001/H11002 mice. The Npt2b protein levels in the distal intestine were significantly decreased in Npt2b/H11001/H11002 mice compared with Npt2b/H11001/H11001 mice. At 4 wk (28 days) of age, Npt2b/H11001/H11002 mice showed hypophosphatemia and hypophosphaturia, suggesting impaired intestinal Pi transport and activation of renal Pi reabsorption. In contrast, at 20 wk, Npt2b/H11002/H11002 mice did not show hypophosphatemia. At 4 wk, the body demand for Pi is markedly increased and Pi from the diet is absorbed.
through the intestine to support skeletal growth (41). In the present study, the role of Npt2b in the weaning animals appeared to be more important than that in adult mice (20 wk). Indeed, the levels of intestinal Npt2b protein are significantly increased in the suckling and weaning periods than in the adult stages (4) (Segawa H, Hanabusa E, Kaneko I, Miyamoto K, unpublished observations). Xu et al. (41) demonstrated that in rat small intestine, NaPi-IIb (Npt2b) gene expression decreased with age, and this observation correlates well with functional studies. Thus NaPi-IIb expression contributes to the ontogenic changes in intestinal Pi absorption.

In addition, Sabbagh et al. (35) reported the temporal effects on Pi homeostasis after gene ablation (conditional Npt2b-KO) analyzed over 10 wk after tamoxifen treatment. In the adult animals, differences in serum Pi levels were not detected between wild-type and conditional Npt2b-KO mice. Although Sabbagh et al. did not discuss the phenotypes of weaning conditional Npt2b-KO mice, adult conditional Npt2b-KO mice did not show hypophosphatemia (35). These findings indicate that despite an apparent reduction in intestinal Pi absorption, serum Pi is maintained in the conditional Npt2b-KO animals via reduced urinary excretion (35). In contrast, in weaning animals, the achievement of a positive external balance is dependent on intestinal absorption and the ability of the kidneys to reclaim filtered Pi. Little Pi is lost in the feces, reflecting its extensive absorption, a process stimulated by dietary Pi deprivation and 1,25(OH)2D3. Weaning Npt2b+/-mice showed increased plasma 1,25(OH)2D3 levels. The Npt2b protein levels, however, were significantly lower in Npt2b+/-mice than in Npt2b+/- mice. These observations suggest that in Npt2b+/-mice, Npt2b cannot be upregulated by stimulation with 1,25(OH)2D3 or hypophosphatemia (9, 34, 38, 41).

Hyperphosphatemia is an important consequence of chronic kidney disease (CKD) (13, 14, 25). Decreasing plasma Pi concentrations is believed to be critical in the management of patients with CKD, especially those on dialysis (13, 14, 25). Recently, Marks et al. (23) used an in situ intestinal loop technique to determine intestinal phosphate absorption in the 5/6 nephrectomy rat model of chronic renal failure (CRF) under disease conditions and reported that Pi absorption in the duodenum and jejunum is not significantly altered, regardless of dietary Pi intake level. These data indicate that in CRF, unlike in the kidney, Pi transport across the small intestine is not reduced (23). This makes intestinal Pi absorption a potential target for the prevention and treatment of hyperphosphatemia (23). Npt2b might thus be a primary target for the development...
of inhibitors of hyperphosphatemia. In the present study, we examined the role of Npt2b in preventing hyperphosphatemia in mice with adenine-induced renal failure. Mice with adenine-induced renal failure showed hyperphosphatemia and high levels of plasma creatinine. Npt2b+/- mice had lower plasma Pi and creatinine levels, suggesting that reduced intestinal Npt2b prevents hyperphosphatemia and the progression of renal failure in this mouse model. Indeed, reductions in Pi intake have been linked to progression of proteinuria, stabilization of glomerular filtration rate, and, in some reports, improved survival in animal models of severe renal failure (1, 2, 7, 12, 15, 18, 19, 22).

Finally, in the present study, we demonstrated that weanling Npt2b+/- mice show hypophosphatemia and hypophosphaturia. Intestinal Npt2b protein levels were significantly decreased in Npt2b+/- mice compared with those in Npt2b+/+ mice. In Npt2b+/- mice with adenine-induced renal failure, plasma Pi levels were significantly decreased compared with those in Npt2b+/+ mice. These data also suggest that Npt2b is an important therapeutic target for the prevention of hyperphosphatemia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


