J. Biochem. 121, 50-55 (1997)

Acute Regulation by Dietary Phosphate of the Sodium-Dependent Phosphate Transporter (NaP_i-2) in Rat Kidney[1]

Kanako Katai,* Hiroko Segawa,* Hiromi Haga,* Kyoko Morita,* Hidekazu Arai,* Sawako Tatsumi,* Yutaka Taketani,* Ken-ichi Miyamoto,*,[2] Setsuji Hisano,† Yoshihiro Fukui,† and Eiji Takeda*

*Departments of *Clinical Nutrition and †Anatomy, School of Medicine, Tokushima University, Kuramoto-cho 3, Tokushima 770*

Received for publication, August 5, 1996

Alteration of the dietary intake of phosphate (P_i) leads to rapid changes in renal P_i transport activity. The present study, examined the underlying cellular mechanisms of the rapid regulation, with special reference to renal P_i cotransporter. Rats were fed either a low-P_i (0.02%) diet (CLP rats), the low-P_i diet followed by a high-P_i (1.2%) diet (AHP rats), or a normal (0.6%) diet (control rats). Na^+-dependent P_i transport activity in the brush border membrane was significantly increased in CLP rats compared with control rats, and this activity decreased rapidly within 2 h after the change of diet in AHP rats. Kinetic analysis of P_i transport in the AHP rats indicated that the reduction was accompanied by a decrease in the apparent V_{max} for Na^+-dependent P_i uptake. Northern blot analysis showed no difference in the abundance of NaP_i-2 mRNA of the kidney between AHP and CLP rats. In contrast, Western blot analysis of renal brush border membrane proteins of AHP rats indicated a significant decrease in the abundance of NaP_i-2 protein as compared with CLP rats. Immunoreactive signals for NaP_i-2 were detected in lysosomal fractions of AHP and CLP rats. Immunohistochemical analysis showed that, NaP_i-2 immunoreactivity in AHP rats was largely reduced in the apical membrane of the proximal tubular epithelial cells. Neither cycloheximide nor actinomycin D affected high-P_i-induced reduction of NaP_i-2 protein in the brush border membrane of AHP rats, indicating that *de novo* protein synthesis of an unidentified regulator protein was not involved in the mechanism of this reduction. In contrast, treatment with colchicine, which disrupts microtubulers, abolished the effect of high-P_i diet on NaP_i-2 expression. These results suggested that rapid endocytotic internalization of NaP_i-2 may occur specifically in the brush border membrane following an acute increase in dietary P_i intake.

Key words: diet, kidney, lysosome, phosphate, transporter.

Membrane protein recycling by endocytosis and exocytosis can be a rapid process (*1, 2*). Studies on renal handling of proteins have shown that the proximal tubule is the major site for reabsorption of filtered proteins and that the initial step of reabsorption is endocytosis at the apical membrane of tubular epithelial cells (*2*). Hydrogen ion secretion is controlled by the recycling of vesicles carrying proton pumps to and from the plasma membrane (*3*). The recycling process in some cells can be regulated by hormones. Vasopressin stimulates the insertion of water channels into the luminal membrane of the renal collecting tubule and urinary bladder (*4*). Insulin stimulates the translocation of glucose transporters (GLUT4) from an intracellular compartment to the plasma membrane in fat cells (*5*). The recycling of membrane transport proteins appears to be coupled with very rapid up- or down-regulation of the capacity of the apical brush border membrane for solute transport (*1*).

In vivo the kidney must respond quickly to alteration in the extracellular environment in order to maintain body fluid and electrolyte homeostasis. Thus, the reabsorptive capacity of the epithelial cells lining the urinary tubule is constantly altered. A rapid change in the dietary intake of P_i is known to alter the Na-P_i transport activity in the apical membrane of renal epithelial cells (*6, 7*). Recently, the cDNA for a Na-P_i transporter protein (NaP_i-2) of rat kidney cortex was identified by expression cloning (*8*). To elucidate cellular mechanisms of acute regulation of NaP_i-2 to alterations in dietary P_i, we analyzed NaP_i-2 protein and mRNA levels by using polyclonal antibodies raised against NaP_i-2 and an encoding cDNA fragment. Results suggest that NaP_i-2 is regulated by endocytosis at the apical membrane in the proximal tubule.

MATERIALS AND METHODS

Animals and Diets—Wistar strain rats (200 g) purchased from SLC, Shizuoka, were kept in plastic cages and

[1] Supported by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, and the Salt Science Research Foundation.

[2] To whom correspondence should be addressed. Phone: +81-886-31-3111 (Ext. 2527), Fax: +81-886-33-7094, e-mail: miyamoto@nutr.med.tokushima-u.ac.jp

received a semisynthetic diet low in P_i (0.02%) only during a 2-h feeding period (between 11:00 AM and 1:00 PM) for 10 days (*9*). On the eleventh day, one group of rats was fed a diet containing a high percentage (1.2%) of P_i instead of the low-P_i diet (AHP rats), while another group continued to receive the same low-P_i diet (CLP rats). Control animals were fed a diet containing 0.6% P_i for the same period of 11 days. After the final feeding, the kidneys were rapidly removed from rats under pentobarbital anesthesia and divided into halves. One half of each kidney was used for RNA isolation, the other half for isolation of brush border membrane vesicles (BBMVs).

Administration of Actinomycin D, Cycloheximide, or Colchicine to Rats—Cycloheximide, an inhibitor of *de novo* protein synthesis (0.5 mg/kg of body weight), actinomycin D, an inhibitor of transcription (0.1 mg/kg of body weight), or colchicine (1 mg/kg of body weight) was injected intraperitoneally 1 h before feeding of the high-P_i diet. Since administration of these reagents leads to decreased food consumption, in this experiment we used rats which had been fed a sufficient amount of experimental diet (8-10 g in wet weight).

Preparation of BBMVs and Transport Measurements—BBMVs were prepared from the rat kidney by the Ca^{2+} precipitation method as previously described (*10*), and the purity of the preparation was assessed by the measurement of leucine aminopeptidase, (Na^+K^+) ATPase and cytochrome-*c* oxidase (*11*). Uptake of radiolabeled P_i was measured by rapid filtration (*11*). Incubation was initiated by the addition of 10 μl of the vesicle suspension to 100 μl of incubation solution [100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.4), and 0.1 mM KH_2PO_4]. Transport was terminated by rapid dilution with 3 ml of ice-cold stop solution (100 mM mannitol, 20 mM Hepes/Tris, 0.1 mM KH_2PO_4, 20 mM $MgSO_4$, and 100 mM choline chloride). The reaction mixture was immediately transferred to a premoistened filter (0.45 μm) maintained under vacuum.

Northern Blot Analysis—Total RNAs were isolated from the kidney by extraction with acid guanidine thiocyanate/phenol/chloroform using the method of Chomczynski *et al.* (*12*). The RNAs were denatured by heating at 70°C in 10 mM Mops, pH 7.0, containing 5 mM sodium acetate, 1 mM EDTA, 2.2 M formaldehyde, and 50% (v/v) formamide for 5 min, and subjected to electrophoresis in 1.5% agarose gel containing 2.2 M formaldehyde. Resolved RNA was transferred to Hybond-N membrane (Amersham) and covalently cross-linked by exposure to UV light. Hybridization was performed in a solution containing 50% formamide, 5× SSPE, 2×Denhardt's solution, and 1% SDS. The membranes were exposed by use of a Fuji BAS-2000 system. NaP_i-2 cDNA probe (2.4 kb) was obtained from a rat kidney cDNA library (*8*). The internal standard was GAPDH cDNA.

Generation of Peptide-Antibody and Western Blotting—An antibody was raised against a peptide that represented amino acid sequences (Leu-Ala-Leu-Pro-Ala-His-His-Asn-Ala-Thr-Arg-Leu) in the C-terminal region of the Na-P_i-cotransporter (321-331), corresponding to a putative C-terminal intracellular domain (*8*). An N-terminal cystein residue was introduced for conjugation with keyhole limpet hemocyanine (KLH, Sigma, St Louis, MO) using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS). The conjugates (100 μg of peptide) were mixed with Freund's complete adjuvant and injected sc into rabbits 4 times at 2-week intervals. In the affinity purification by use of a gel column (Cellulofine, Seikagaku-Kogyo, Tokyo), the antiserum (10 ml) was loaded on the gel coupled with the peptide and the NaP_i-2 antibodies were eluted with 0.1 M glycine-HCl (pH 2.5), neutralized, dialyzed, and stored at −80°C.

Rat renal proximal BBMVs were prepared as described above and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) in the presence of protease inhibitors. The separated proteins were transferred electrophoretically to a nylon filter as described by Towbin *et al.* (*13*).

Immunohistochemistry—Under pentobarbital anesthesia, CLP and AHP rats were transcardially perfused with saline followed by 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). Kidneys were removed, immersed in the fixative for 15 h and processed for paraffin sections. After microwave irradiation (for 10 min in 10 mM citrate buffer, pH 6.0) and treatment with hydrogen peroxide, the sections were incubated with the NaP_i-2 antibody (1:2,000) overnight at 4°C. NaP_i-2 immunoreactivity was detected with avidin-biotin-peroxidase complex (Vector, Elite Kit). To verify the specificity of immunoreaction, parallel staining was carried out with the antibody that had been mixed with the antigen peptide (50 μg/ml) (*14*).

Isolation of Lysosomal Fractions—CLP and AHP animals were killed by decapitation. After perfusion with ice-cold 0.25 M sucrose solution to remove blood, kidneys were excised, minced well with scissors and suspended in 4 volumes of cold 0.25 M sucrose. The suspension was then homogenized, first with 20 strokes by hand in a Dounce homogenizer, followed by 2 strokes in a Potter-Elvehjem homogenizer rotating at 800 rpm. The resulting 20% homogenate was centrifuged for 5 min at 340×*g*. The supernatant was again centrifuged under the same conditions. Next, the supernatant was incubated at 37°C for 5 min in the presence of 1 mM $CaCl_2$, then layered onto iso-osmotic (0.25 M sucrose) Percoll at a density of 1.08 g/ml. This was followed by centrifugation at 60,000×*g* in a Hitachi RP 30 rotor at 2°C for 15 min (*15*), which generated density gradients ranging from 1.06 to 1.14 g/ml. The lysosomes were recovered from the denser fractions of the gradient heterogeneously dispersed above 1.09 g/ml by the distribution of arylsulfatase. These fractions containing arylsulfatase were pooled and centrifuged for 1 h at 100,000×*g*. After centrifugation, the broad turbid layer (lysosomal fraction) in the middle of the tube was carefully collected with a Pasteur pipette. The lysosomal fraction was diluted with two volumes of 0.25 M sucrose solution. The diluted suspension was centrifuged at 10,000×*g* for 30 min to obtain a pellet covered by a fluffy layer. The pellet and fluffy layer were carefully collected, suspended in 0.25 M sucrose solution and centrifuged at 10,000×*g* for 30 min. The pellet was washed twice by centrifugation under the same conditions, which effectively removed the contaminating Percoll. The washed pellet was finally suspended in 500 μl of 0.25 M sucrose solution and used as the purified lysosomal fraction (*15*). The lysosomal fraction obtained by our method was enriched more than 120-fold in terms of the marker enzymes (arylsulfatase and cathepsins B and H) with a yield of 25% (*15, 16*).

Statistical Analysis—Statistical evaluation was accom-

plished by the Student t-test or by the ANOVA program. All results are expressed as mean±SEM.

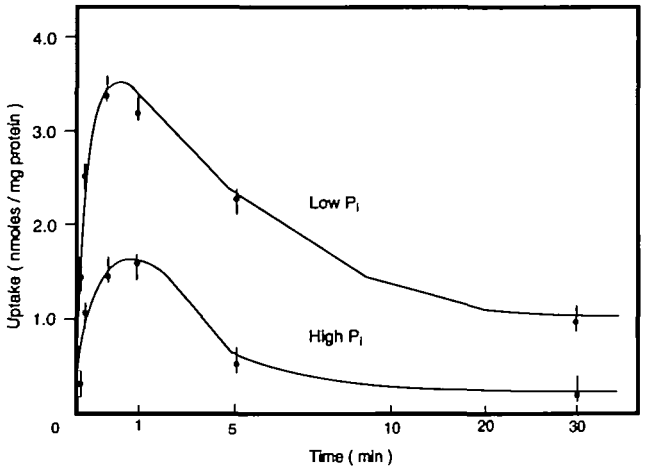

Fig. 1. **Phosphate transport activity in BBMVs isolated from kidney of CLP rats.** BBMVs were obtained from CLP (4 h after start of feeding) and normal rats. They were incubated for 1, 5, 10, and 30 s and 1, 10, 20, and 30 min. The filter washed in a Na-free P_i solution and was transferred to a scintillation solution. Values are presented ± SEM. $n=6$.

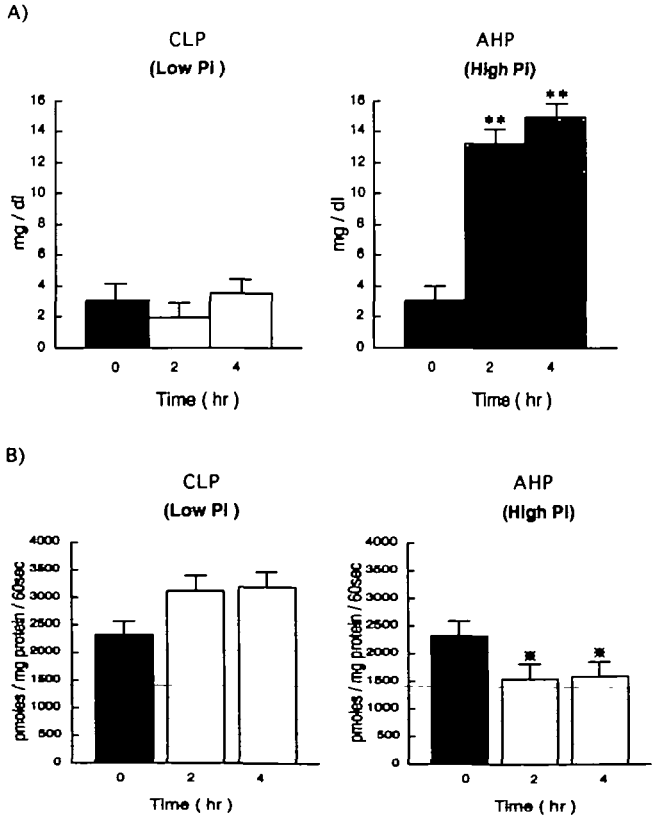

Fig. 2. **Relationship between P_i transport activity and plasma P_i concentration in CLP and AHP rats.** (A) Changes of plasma P_i concentration in CLP and AHP rats. Data represent means±SEM, $n=6$. $^{**}p<0.001$. (B) P_i uptake in BBMVs in CLP and AHP rats 2 and 4 h after the start of feeding. Data represent means±SEM, $n=6$. $^{*}p<0.01$.

RESULTS

1) Effects of Dietary P_i Intake on Na^+-Dependent P_i Transport into BBMVs—In CLP and control rats, the uptake of P_i by the BBMVs as a function of incubation time showed an overshoot in the presence of a Na^+ gradient (Fig. 1). Na^+-dependent P_i uptake at 1 min increased approximately 2.3-fold in CLP rats as compared with the control animals ($p<0.01$). In contrast, Na^+-P_i transport activity in the brush border membrane of the AHP rats exhibited 44 and 42% decreases compared with CLP rats within 2 h ($^{*}p<0.01$) and 4 h ($^{*}p<0.01$), respectively (Fig. 2B). However, there was no change in the Na^+-dependent glucose and amino acid transport activities of the AHP rats (data not shown). As shown in Fig. 2A, plasma P_i levels were low in CLP animals, but rose sharply in AHP rats after they had been fed the high-P_i diet. In addition, kinetic analysis on the acute regulation of Na^+-dependent P_i transport in BBMVs showed that the decrease in Na^+-dependent P_i uptake is due to the change of V_{max}, not K_m (Table I).

2) Effect of Dietary P_i Intake on NaP_i-2 Expression—As shown in Fig. 3, NaP_i-2 mRNA levels were significantly increased in CLP rats. In contrast, levels of the mRNA were unchanged in AHP rats 2 and 4 h after they had been the

TABLE I. **Kinetic parameters of P_i transport in renal brush border membrane of CLP and AHP rats 4 h after the start of feeding.**

	V_{max} (nmol/mg/10 s)	K_m (mM)
CLP rats	2.35±342	0.12±0.02
AHP rats	1.24±166[a]	0.11±0.03

[a] $p<0.01$, $n=6$.

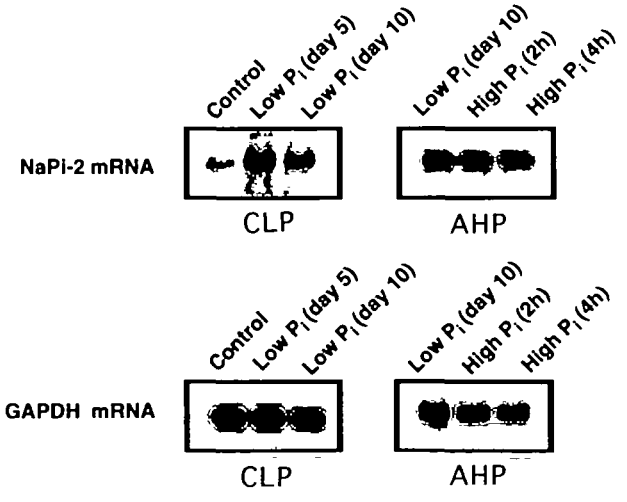

Fig. 3. **Levels of NaP_i-2 mRNA in rats in CLP or AHP rats.** Total RNA was purified from the renal cortex of CLP and AHP rats. A 20-μg portion was loaded onto 1.2% formamide gel and transferred to a nylon membrane. The DNA probe was prepared as described previously (*21*) and labeled by the random primed method. The filters were washed with 0.1×SSPE and 0.1% SDS at 65°C for 30 min. Autoradiography was then performed (Fuji BAS 2000, Tokyo). RNA samples were obtained from rats fed a low-P_i diet for 5 and 10 days (CLP rats) and 2 and 4 h after they had been fed the high-P_i diet (AHP rats). Control represents the rats before feeding the low-P_i diet.

high-P_i diet. In Western blot analysis, the NaP_i-2 antibody recognized a large-molecular-mass complex at 157 and 90 kDa in kidney cortex BBMVs. The latter band was intense, probably representing the predominant forms of this transporter in rat kidney. All bands were undetectable when the antibody was mixed with antigenic peptide before use (Fig. 4A), suggesting that the antibody specifically recognizes NaP_i-2 in rat kidney. In CLP rats, densitometric analysis of NaP_i-2 protein showed that the intensity of these bands was significantly increased by about 3.5-fold over that in control rats. In AHP rats, the amount of NaP_i-2 proteins (90 kDa) was significantly decreased by about 5-fold compared with CLP rats (Fig. 4B).

We determined whether NaP_i-2 protein from BBMVs was sequestered into the lysosomal fraction. As shown in Fig. 5, Western blot analysis showed 35- and 17-kDa bands to be present in a highly purified lysosomal fraction. In AHP rats, the immunoreactivity of these bands was significantly increased 2 and 4 h after they had been fed the high-P_i diet. However, densitometric analysis showed that the relative increment of immunoreactive bands in the AHP rats was not different from that of CLP rats.

Immunohistochemical analysis of NaP_i-2 in the kidney of CLP rats showed that positive staining is highly restricted to the brush border membrane of tubular epithelial cells throughout the kidney cortex (Fig. 6, A and B). In AHP rats the NaP_i-2 staining was much less intense than in CLP rats (Fig. 6, C and D). This was more prominent in the superficial and midcortical regions than in the juxtamedullary region of the cortex.

3) Effects of Drugs on the Expression of NaP_i-2 Protein—To determine whether the reduction of NaP_i-2 immunoreactivity in AHP rats is due to *de novo* synthesis of an unidentified regulator protein, the rats were injected intraperitoneally with either cycloheximide or actinomycin D prior to feeding with the high-P_i diet. However, these treatments had no effect on the high-P_i-induced reduction of NaP_i-2 protein, as shown in Fig. 7.

To assess whether the microtubule participates in the down-regulation of NaP_i-2 expression after ingestion of the high P_i diet, AHP rats were pretreated with intraperitoneal administration of colchicine. The pretreatment completely

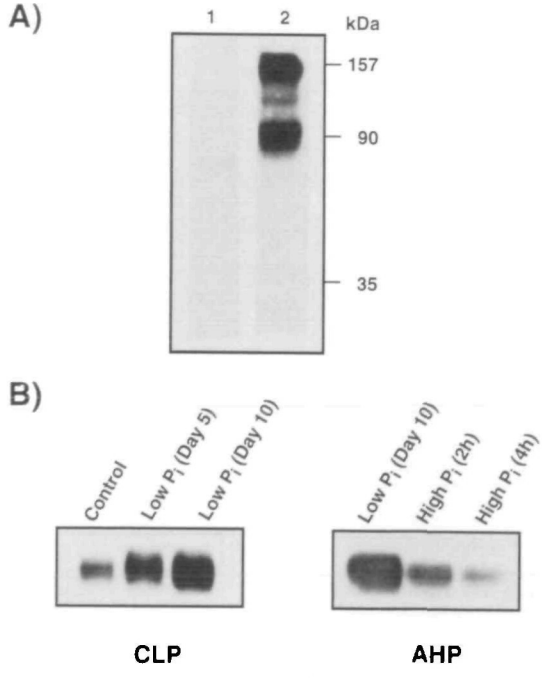

Fig. 4. **Western blot analysis of NaP_i-2 in the brush border membrane of the rat renal cortex.** (A) Western blot analysis of BBMVs obtained from normal rat kidney. Renal BBMVs were prepared as described previously (*10*). Anti-NaP_i-2 specific antibody was purified by affinity column chromatography. A 20-μg portion of renal BBMVs proteins was solubilized at 100°C for 3 min in the absence of β-mercaptoethanol, fractionated by 10% SDS-PAGE, and electrotransferred onto nitrocellulose membrane. The filter was incubated with anti-NaP_i-2 antibody (lane 2). Parallel staining was carried out with the antibody mixed with the antigen peptide (50 μg/ml) (lane 1). (B) Levels of NaP_i-2 protein in CLP and AHP rats. A 30-μg portion of purified BBMVs obtained from the same animals as in Fig. 3, was subjected to 10% SDS-PAGE. Anti-NaP_i-2 antibody was purified as described in the text. Reaction signals were detected by use of Enhanced Chemiluminescence (ECL, Amersham).

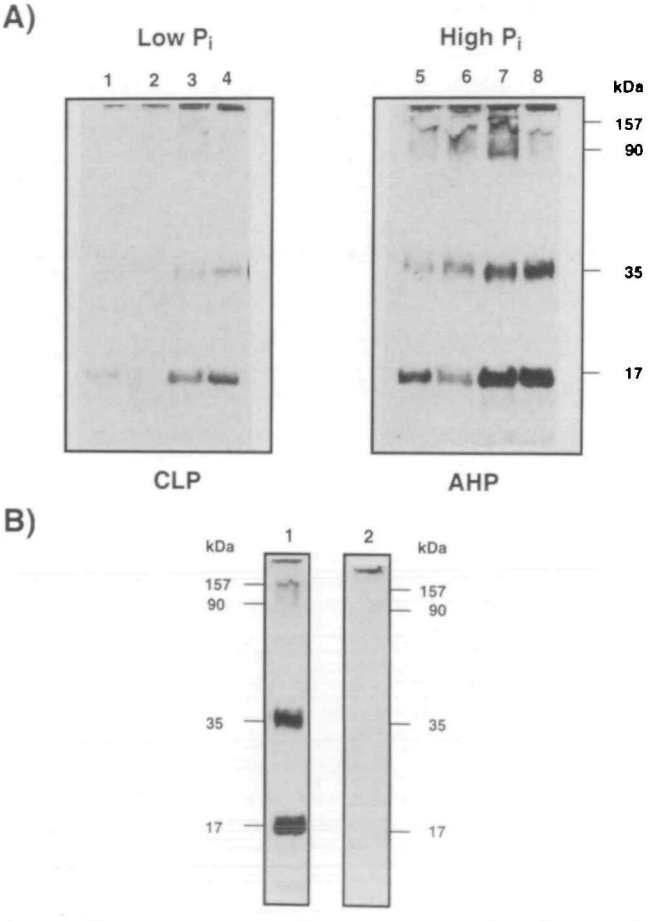

Fig. 5. **NaP_i-2 immunoreactive protein in purified lysosomal fraction.** The presence of NaP_i-2-immunoreactive signals was detected by Western blotting. The purified lysosomal fraction (10 mg/100 μl) was fractionated by 10% SDS-PAGE, and transferred to a nitrocellulose membrane. (A) Lanes 1-4: purified lysosomal fractions obtained from rats 0, 1, 2, and 4 h after they had been fed the low-P_i diet (CLP rats); lanes 5-8: purified lysosomal fractions obtained from rats 0, 1, 2, and 4 h after they had been fed the high-P_i diet (AHP rats). (B) Half of the membrane containing the purified lysosomal fractions (A, lane 8) was incubated with the antibody mixed with the synthetic peptide (50 μg/ml) (lane 2) and the other half was incubated with unabsorbed NaP_i-2 antibody (lane 1).

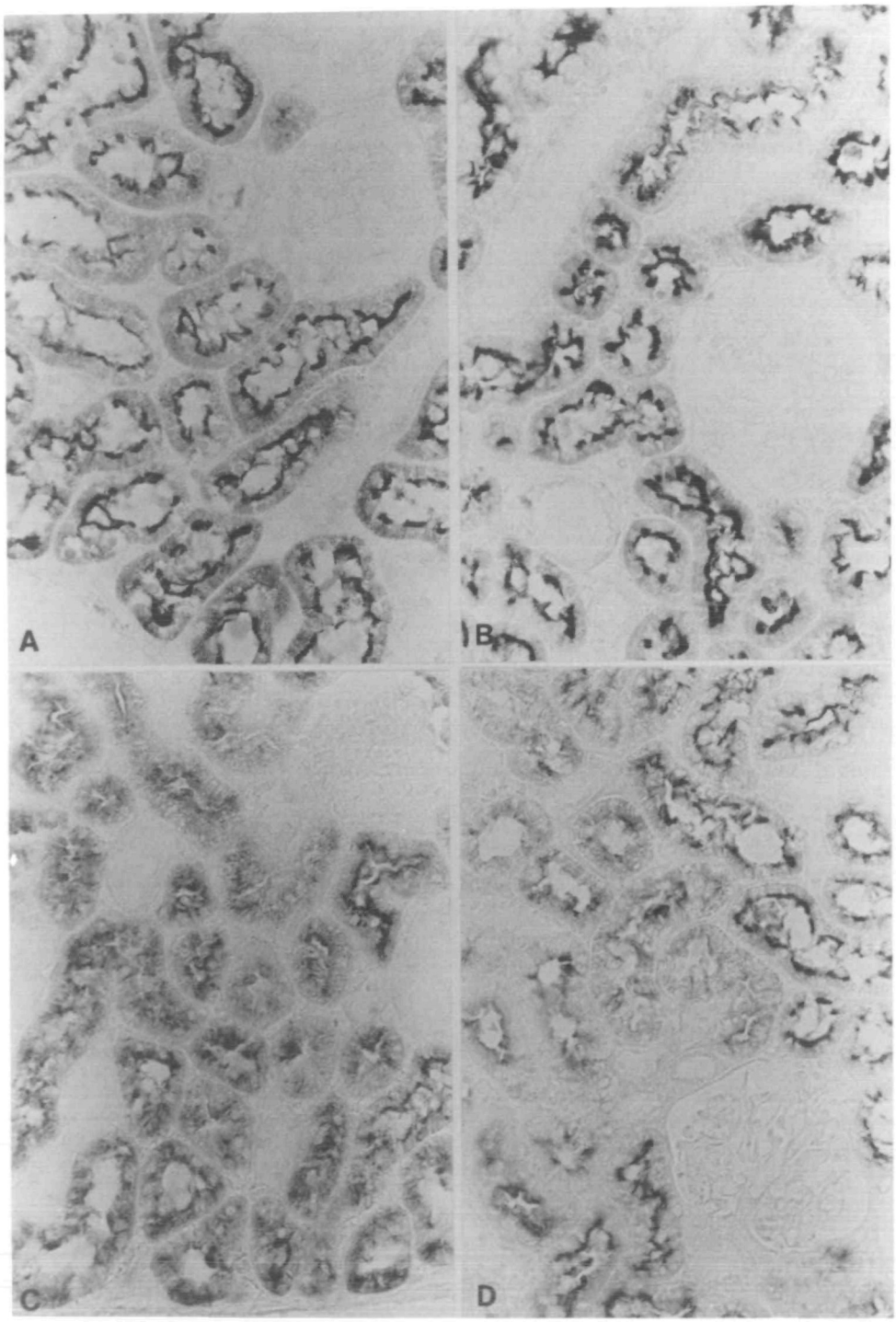

Fig. 6. **Localization of NaP_i-2-immunoreactivity in rat renal cortex.** Intense positive reaction was detected mainly in the superficial and midcortical than the juxtamedullary nephrons. (A) The superficial region of the kidney of a CLP rat at 4 h. (B) The midcortical region in the same rat. (C) The superficial region of the kidney of an AHP rat at 4 h. (D) The midcortical region in the same rat.

abolished the positive effect of high-P_i intake on NaP_i-2 expression (Fig. 7).

DISCUSSION

In the rats chronically fed the low-P_i diet, a significant increase was observed in Na^+-dependent P_i transport activity, probably due to the elevated expression of NaP_i-2 mRNA and protein. These observations are consistent with results of other workers (*17, 18*). In rats switched from the low-P_i to the high-P_i diet (AHP rats), the P_i transport activity was shown to decrease down to the level of control animals immediately after switch. The reduction of the activity seems to be independent of PTH, because no changes were observed in serum PTH level in those animals (data not shown).

PTH stimulates the overall rate of the constitutive endocytotic pathway and specifically targets the Na-dependent P_i transporters for their endocytotic removal from the brush border membrane (*1, 16*). Kempson *et al.* postulated that PTH action may be explainable by changes of the expression of NaP_i-2 protein in the renal brush border

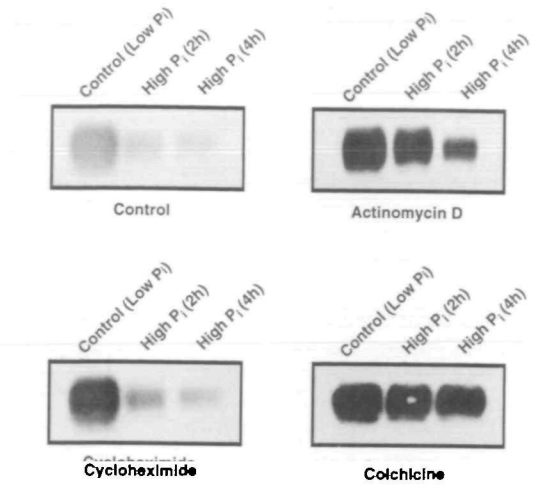

Fig. 7. **Effects of the administration of actinomycin D, cycloheximide, and colchicine in NaP_i-2 protein in AHP rats.** Cycloheximide, actinomycin D, or colchicine was administrated to rats 1 h before they were fed the high-P_i diet. The animals were killed and BBMVs were purified. Western blot analysis was performed as described in Fig. 4. The relative amount of NaP_i-2 protein was determined by densitometric analysis.

membrane. It is possible that PTH induces an endocytotic withdrawal of NaP_i-2 protein from the brush border membrane to a cytoplasmic pool, or that PTH inhibits expression of NaP_i-2 mRNA (*16*). Our results suggest that acute regulation of NaP_i-2 induced by the high-P_i diet is mediated through an endocytotic pathway and is unrelated to the changes of mRNA level, suggesting that the mechanisms of down-regulation by the high-P_i diet are different from those of PTH. Indeed, in thyroparathyroidectomized rats, the acute regulation of NaP_i-2 is retained after ingestion of a high-P_i diet, but to a less extent than in intact animals (K. Katai, unpublished observations).

Colchicine, which disrupts the microtubules, prevented the reduction of NaP_i-2 protein in AHP rats. In addition, we demonstrated that NaP_i-2-immunoreactive materials exist in lysosomal fractions of CLP and AHP rats, suggesting the translocation of NaP_i-2 from the brush border membrane to lysosomes. It is well known that endocytosis followed by lysosomal degradation requires an intact microtubule network (*1*), which may regulate the activity and/or the number of transport proteins in the cell membrane (*1, 7, 19*). The potential role of the microtubules in the delivery of reserve transporters has been proposed for other cell systems (*19*). For example, colchicine simultaneously inhibits the PTH-dependent increase in Ca^{2+} transport and exocytosis in distal tubular cells (*20*), and it further suppresses the secretin-induced increase in HCO_3^- secretion and membrane fusion of cytoplasmic vesicles in pancreatic ducts (*19*). Thus, microtubules may play a key role in the trafficking of vesicles containing NaP_i-2 from its docking sites in the cell membrane.

Finally, our data suggest that NaP_i-2 is internalized from the brush border membrane and translocated into lysosomes shortly after feeding of the high P_i diet. The mechanism of internalization is independent of *de novo* protein synthesis but controlled in a colchicine-sensitive manner.

REFERENCES

1. Brown, D. and Stow, J.L. (1996) Protein trafficking polarity in kidney epithelium: From cell biology to physiology. *Physiol. Rev.* **76**, 245-297
2. Coudrier, E., Kerjaschki, D., and Louvard, D. (1988) Cytoskeleton organization and submembranous interactions in intestinal and renal brush borders. *Kidney Int.* **34**, 309-320
3. Schwartz, G.J. and Al-Awqati, Q. (1985) Carbon dioxide causes exocytosis of vesicles containing H^+ pumps in isolated perfused proximal and collecting tubules. *J. Clin. Invest.* **75**, 1638-1644
4. Verkman, A.S. (1992) Water channels in cell membranes. *Annu. Rev. Physiol.* **54**, 97-108
5. Kono, T., Suzuki, K., Dansey, L.E., Robinson, F.W., and Blevins, T.L. (1981) Energy-dependent and protein synthesis-independent recycling of the insulin-sensitive glucose transport mechanism in fat cells. *J. Biol. Chem.* **256**, 6400-6407
6. Murer, H. and Biber, J. (1992) Renal tubular phosphate transport in *The Kidney: Physiology and Pathophysiology*, 2nd ed. (Seldin, D.W. and Giebisch, G., eds.) pp. 2481-2509, Raven Press, New York
7. Berndt, T.J. and Knox, F.G. (1992) Renal regulation of phosphate excretion in *The Kidney: Physiology and Pathophysiology*, 2nd ed. (Seldin, D.W. and Giebisch, G., eds.) pp. 2511-2531, Raven Press, New York
8. Magagnin, S., Werner, A., Markovich, D., Sorribas, V., Stange, G., Biber, J., and Murer, H. (1993) Expression cloning of human and rat renal cortex Na/P_i cotransport. *Proc. Natl. Acad. Sci. USA* **90**, 5979-5983
9. Stoll, R., Kinne, R., and Murer, H. (1979) Effect of dietary phosphate intake on phosphate transport by isolated rat renal brush-border vesicles. *Biochem. J.* **180**, 465-470
10. Minami, H., Kim, J.R., Tada, K., Takahashi, F., Miyamoto, K., Nakabou, Y., Sakai, K., and Hagihira, H. (1993) Inhibition of glucose absorption by phlorizin affects intestinal functions in rats. *Gastroenterology* **105**, 692-697
11. Nakagawa, N., Arab, N., and Ghisham, F.K. (1991) Characterization of the defect in the Na^+-phosphate transporter in vitamin D-resistant hypophosphatemic mice. *J. Biol. Chem.* **266**, 13616-13620
12. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159
13. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354
14. Hisano, S., Haga, H., Miyamoto, K., Takeda, E., and Fukui, Y. (1996) The basic amino acid transporter (rBAT)-like immunoreactivity in paraventricular and supraoptic magnocellular neurons of the rat hypothalamus. *Brain. Res.* **710**, 299-302
15. Yamada, H., Hayashi, H., and Natori, Y. (1984) A simple procedure for the isolation of highly purified lysosomes from normal rat liver. *J. Biochem.* **95**, 1155-1160
16. Kempson, S.A., Lotscher, M., Kaissling, B., Biber, J., Murer, H., and Levi, M. (1995) Parathyroid hormone action on phosphate transporter mRNA and protein in rat renal proximal tubules. *Am. J. Physiol.* **268**, F784-F791
17. Biber, J., Custer, M., Magagnin, S., Hayes, G., Werner, A., Lotscher, M., Kaissling, B., and Murer, H. (1996) Renal Na/P_i-cotransporters. *Kidney Int.* **49**, 981-985
18. Werner, A., Kempson, S.A., Biber, J., and Murer, H. (1994) Increase of Na/P_i-cotransport encoding mRNA in response to low P_i diet in rat kidney cortex. *J. Biol. Chem.* **269**, 6637-6639
19. Mills, J.W. and Mandel, L.J. (1994) Cytoskeletal regulation of membrane transport events. *FASEB J.* **8**, 1161-1165
20. Bacskai, B.J. and Friedman, P.A. (1990) Activation of latent Ca^{2+} channels in renal epithelial cells by parathyroid hormone. *Nature* **347**, 388-391