Intraperitoneal Administration of Recombinant Receptor-Associated Protein Causes Phosphaturia via an Alteration in Subcellular Distribution of the Renal Sodium Phosphate Co-Transporter

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Megalin is a multifunctional endocytic receptor that is expressed in renal proximal tubules and plays critical roles in the renal uptake of various proteins. It was hypothesized that megalin-dependent endocytosis might play a role in renal phosphate reabsorption. For addressing the short-term effects of altered megalin function, a recombinant protein for the soluble form of 39-kD receptor-associated protein (RAP) was administered intraperitoneally to 7-wk-old mice. Histidine (His)-tagged soluble RAP (amino acids 39 to 356) lacking the amino-terminal signal peptide and the carboxy-terminal endoplasmic reticulum retention signal was prepared by bacterial expression (designated His-sRAP). After the direct interaction between His-sRAP and megalin was confirmed, mice were given a single intraperitoneal administration of His-sRAP (3.5 mg/dose). Immunostaining and Western blot analyses demonstrated the uptake of His-sRAP and the accelerated internalization of megalin in proximal tubular cells 1 h after administration. In addition, internalization of the type II sodium/phosphate co-transporter (NaPi-II) was observed. The effects of three sequential administrations of His-sRAP (3.5 mg/dose, three doses at 4-h intervals) then were examined, and increased urinary excretion of low molecular weight proteins, including vitamin D–binding protein, was found, which is consistent with findings reported for megalin-deficient mice. It is interesting that urinary excretion of phosphate was also increased, and the protein level of NaPi-II in the brush border membrane was decreased. Serum concentration of 25-hydroxyvitamin D was decreased, whereas the plasma level of intact parathyroid hormone was not altered by the administration of His-sRAP. The results suggest that the His-sRAP–induced acceleration of megalin-mediated endocytosis caused phosphaturia via altered subcellular distribution of NaPi-II.


Megalin is a large membrane protein that belongs to the LDL receptor–related protein family (1–3). It functions as a multifunctional endocytic receptor together with cubilin and is expressed in the apical membrane of renal proximal tubules and several other absorptive epithelia (4,5). Ligands that are internalized with megalin are transported from apical clathrin-coated pits to endosomes, where the complexes are dissociated and the ligands are sorted into lysosomes to be degraded (6–8). However, megalin itself is thought to be recycled by re-fusion of endosomes and the apical membrane (6–8).

Findings in megalin-knockout mice have indicated the critical roles of megalin in brain development and the renal uptake of various filtered proteins, including vitamin D–binding protein (DBP), transcobalamin, and thyroglobulin (9). Although approximately 98% of megalin-deficient mice die perinatally as a result of holoprosencephaly, surviving animals exhibit increased urinary excretion of DBP complexed with 25-hydroxyvitamin D (25OHD) and low molecular weight proteins (10–12). Urinary loss of 25OHD results in the reduction of serum 25OHD levels in megalin-knockout mice (11). The 1α-hydroxylation of 25OHD is a critical step for vitamin D to exert its function as an active metabolite, including maintaining the serum calcium levels, and is closely regulated by various factors, including parathyroid hormone (PTH). The 1α-hydroxylation of 25OHD occurs in renal proximal tubular cells, and the uptake of 25OHD together with DBP was previously believed to be an event in the basolateral membrane of the cells, in other words, from the bloodstream. However, megalin-knockout mice revealed that the 25OHD/DBP complex is actually filtered by glomeruli and is subsequently reabsorbed in renal tubular cells across the brush border membrane via megalin-mediated endocytosis (11).

To further examine the function of megalin in the kidneys, kidney-specific megalin-deficient mice have also been generated (13,14). Analysis of those mice clearly indicated the involvement of megalin in the renal uptake of 25OHD, as well as...
proteins with relatively low molecular weight. In addition, the animals exhibited impaired trafficking of a renal Na+/Pi co-transporter (NaPi-II), suggesting the involvement of megalin in phosphate metabolism (14).

Although it is still not clear which human disease is caused directly by a defect in megalin, some diseases, including Dent’s disease and some forms of renal Fanconi syndrome, are reported to be associated with secondary malfunction of megalin (15,16). Reduced apical expression of megalin in the proximal tubules, as well as reduced levels of megalin detected in urine, was demonstrated in a mouse model of Dent’s disease (17,18). In addition, some medicines such as antibiotics (aminoglycosides) and anticancer drugs exert a nephrotoxic effect and cause malfunction of renal tubular cells (19,20). The excretion of these polybasic drugs interferes with megalin function. For fully understanding the roles of megalin, especially in such pathologic conditions, it is necessary to establish appropriate animal models that allow us to elucidate the acute effects of impaired megalin function.

Receptor-associated protein (RAP) is a 39-kD protein that is critical for the normal processing of megalin in various cells, including proximal tubular cells (21). RAP contains an endoplasmic reticulum (ER) retention signal at the carboxyl terminus and is mainly located in the ER (21). Indeed, RAP deficiency generated by a gene-targeting technique is associated with reduced expression levels of megalin in the plasma membrane (22). When added to extracellular fluid, however, RAP binds to the ligand-binding domain of megalin and inhibits the binding of all of the other ligands (23). Hence, the administration of RAP to animals may model an acute loss of megalin function.

In this study, we hypothesized that megalin-dependent endocytosis might play a role in renal phosphate reabsorption and examined the short-term effects of intraperitoneal administration of a recombinant soluble form of RAP in mice. It is interesting that administration of soluble RAP caused phosphaturia, as well as increased urinary excretion of low molecular weight proteins, including DBP. In addition, administration of RAP affected the subcellular distribution of NaPi-II in proximal tubular cells. These results suggest the involvement of megalin in phosphate reabsorption in the kidneys.

Materials and Methods

Preparation of a Recombinant Soluble Form of RAP

To construct the bacterial expression vector for the soluble form of murine RAP, we performed PCR-based cloning of the cDNA using total RNA extracted from mouse kidney. Reverse transcription (RT) was carried out using random hexamer (Promega, Madison, WI) and SuperScript II (Invitrogen, Carlsbad, CA), followed by PCR using the primers mRAP-F (sense 5’-AGAGGAGAGATGGCCCTGGAAG-3’) and mRAP2c-R (antisense 5’-AGAGATCCCTACCCGACCCCTTCGAGACCTGTAGAC-3’). The amplified product was cloned to pT7-Blue vector (Novagen, Madison, WI). The fragment excised by Scal/EcoRI digestion was then cloned to the BamHI site in frame into the pET15b vector (Novagen) carrying an amino-terminal His-tag sequence. The resulting plasmid pET15b-sRAP encodes murine RAP corresponding to the amino acids (a.a.) 39 to 356, lacking the amino-terminal signal peptide and the carboxy-terminal ER retention signal (HNEL as a one-letter amino acid description). The recombinant His-tagged soluble form of RAP (His-sRAP, 342 a.a.) protein was then expressed in Escherichia coli BL21 (DE3). The expressed His-sRAP protein was purified using Chelating Sepharose Fast Flow (Amersham Biosciences, Piscataway, NJ). The size (approximately 40 kD) and the purity of the recombinant protein were confirmed by SDS-PAGE followed by Coomassie blue staining.

Antibodies

A polyclonal antibody was raised against megalin by preparing a recombinant protein corresponding to a 195 a.a. in the carboxy-terminal intracellular domain of mouse megalin as an antigen and immunizing white rabbits (16). In addition, another polyclonal antibody was raised against a peptide representing an a.a. sequence (MMSYSERLLG-PAYSP) in the amino-terminal region of NaPi-II according to a previous report (24). The antibodies against His-tag were purchased from Santa Cruz (His-probe; Santa Cruz, CA) and Roche (Anti-His6-Peroxidase; Mannheim, Germany). For detecting DBP, an antibody against Gc-globulin was obtained from Dako Cytomation (Carpinteria, CA).

Ligand Blotting

Membrane fractions prepared from mouse kidneys following the previous report (25) were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes (BioRad, Hercules, CA). After blocking, the membrane was incubated with 0.2 mg/ml His-sRAP in a binding buffer (50 mM Tris [pH 8.0], 80 mM NaCl, 2 mM CaCl2, 0.5 mM MgCl2, 1% BSA, and 0.1% Triton-X100). After washing in a washing buffer (2 mM CaCl2 and 0.5 mM MgCl2 in Tris-buffered saline), the membrane was immunoblottedted with an anti-His, antibody conjugated with peroxidase (Roche). Signals were detected using an enhanced chemiluminescence (ECL) system (Amersham Biosciences).

Animal Experiments

Animal protocols were approved by the Institutional Animal Care and Use Committee at Osaka Medical Center and Research Institute for Maternal and Child Health. Male ICR mice (7 wk old) were supplied by Clea Japan (Tokyo, Japan) and maintained under pathogen-free conditions. The experimental protocols are shown in Figure 1. For examining whether intraperitoneally administered His-sRAP was taken up by proximal tubular cells, mice were given a single injection of recombinant His-sRAP (3.5 mg) and then killed before (designated as 0 h) or 1, 2, or 6 h after the injection for use in the histologic kidney analysis (Figure 1a). In another series of experiments, mice were placed in metabolic cages to collect blood and urine samples. After the collection of blood and 20-h urine as pretreatment samples, mice were given intraperitoneal administrations of recombinant His-sRAP (3.5 mg) or a vehicle three times at 4-h intervals (Figure 1b). The 20-h urine samples after the second administration were collected as posttreatment urine samples. After collection of the urine samples, the mice were killed and blood and kidneys were harvested (Figure 1b).

Immunostaining

The kidney specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin, and cut into 5-µm-thick sections. Immunohistochemical staining was performed using the His-probe (Santa Cruz) and antibodies against megalin and NaPi-II. When the antibody against NaPi-II was used, the specimens were pretreated by incubation in 10 mM citric acid buffer (pH 6.5) at 95°C for 10 min. For detecting the signals, an Envision kit/HRP(DAB) (Dako Cytomation) was used.

For immunofluorescence, kidney specimens were snap-frozen in liquid nitrogen, and 4-µm-thick sections were cut. As the primary anti-
Figure 1. Summary of the animal experimental protocols. (a) Male ICR mice (7 wk old) were given a single intraperitoneal administration of the histidine (His)-tagged soluble form of receptor-associated protein (RAP; His-sRAP; 3.5 mg) and were killed at the indicated time points for histologic analyses of the kidneys. (b) His-sRAP (3.5 mg/dose) or PBS as a vehicle was administered intraperitoneally to 7-wk-old mice three times at 4-h intervals. Urine samples (20-h) were collected before the injections and after the second injection. Blood samples were taken before the first injection and at the end of urine sampling after the injection.

bodies, His-probe raised in goat (Santa Cruz) and rabbit polyclonal antibodies against megalin and NaPi-II were used. As the secondary antibodies, Alexa Flour 488 donkey anti-rabbit IgG and Alexa Flour 555 donkey anti-goat IgG were used (Molecular Probes, Eugene, OR).

Preparation of Brush Border Membrane Fraction

The cortical brush border membrane (BBM) fraction was prepared using a differential centrifugation method previously described by Kumar and Prasad (26). All preparative steps were performed at 4°C. The cortices from kidneys were dissected, and a 10% homogenate was prepared in ice-cold 50 mM mannitol buffered with 15 mM HEPES buffer (pH 7.0). MnCl2 was added to a final concentration of 4 mM, and the mixture was incubated at 4°C for 10 min. The suspension was centrifuged at 4000 g for 20 min. The resulting pellet was resuspended in 300 mM mannitol and 25 mM HEPES buffer (pH 6.9). This step was repeated twice, and the resulting BBM pellet was resuspended in 300 mM mannitol and 25 mM HEPES buffer (pH 6.9). Alkaline phosphatase activity was assayed to confirm the quality of the BBM fraction.

Western Blotting

The BBM and non-BBM fractions that contained 10 μg of each protein were subjected to SDS-PAGE and were transferred to polyvinylidene difluoride membranes (Biorad). After blocking, the membranes were incubated with the following primary antibodies: Anti-megalin antibody, His-probe, or anti–NaPi-II antibody. After incubation with the corresponding secondary antibodies, the proteins were visualized using an ECL detection system (Amersham Biosciences).

Analysis of Urine Samples

Concentrations of creatinine and phosphate in the urine samples were determined on an autoanalyzer. The urine samples collected were also subjected to SDS-PAGE on a 10% gel followed by silver staining. For detecting DBP in urine samples, Western blot analysis was performed using an antibody against DBP (Dako Cytomation).

Measurement of Circulating Concentrations of 25OHD and Intact PTH

The serum concentration of 25OHD was determined using an RIA kit (DiaSorin, Stillwater, MN). The plasma concentration of intact PTH was measured using a mouse intact PTH ELISA kit (Immutopics, San Clemente, CA).

Statistical Analyses

Statistical analyses were performed using t test.

Results

Interaction between Recombinant His-sRAP and Megalin

The purified His-sRAP was detected as a single band in SDS-PAGE followed by Coomassie blue staining (Figure 2a). For confirming the direct interaction between recombinant His-sRAP expressed in E. coli and megalin in kidney extracts, a ligand blot analysis was performed as described in the Materials and Methods section. As a result, the antibody against His-tag detected a signal with molecular weight comparable to that of megalin (Figure 2b). When an identical membrane was immunoblotted with the antibody against megalin, a signal of similar size was detected, confirming the direct interaction between His-sRAP and megalin (Figure 2c).

Figure 2. Direct interaction between His-sRAP and megalin. (a) Purified recombinant His-sRAP was subjected to SDS-PAGE, followed by Coomassie blue staining. M, molecular weight marker; R, His-sRAP. Purified His-sRAP was detected as a single band. (b) Ligand blot analysis using His-sRAP. Membrane fractions that were prepared from mouse kidneys were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes. After blocking, the membrane was incubated with 0.2 mg/ml His-sRAP in a binding buffer (50 mM Tris [pH 8.0], 80 mM NaCl, 2 mM CaCl2, 0.5 mM MgCl2, 1% BSA, and 0.1% Triton-X100) for 2 h at room temperature. The membrane then was washed and immunoblotted with an anti-His antibody conjugated with peroxidase, and signals were detected using an ECL system. The asterisk indicates the position of the signal. (c) An identical membrane to (b) was subjected to immunoblotting using an anti-megalin antibody. The asterisk indicates the position of the signal.
Uptake of Recombinant His-sRAP by Proximal Tubular Cells after Intraperitoneal Administration

The antibody against megalin immunoreacted with the BBM of the proximal tubules in untreated mice (Figure 3, 0 h). Because the direct interaction between His-sRAP and megalin was confirmed by the ligand blot analysis described above, we next examined whether intraperitoneally administered His-sRAP was taken up by proximal tubular cells. Male ICR mice were given a single intraperitoneal injection of recombinant His-sRAP (3.5 mg/dose), then killed before (designated 0 h) or 1, 2, or 6 h after the injection for histologic kidney analysis (Figure 1a). In immunohistochemical examination using the His-probe His-sRAP was detected in the subapical area of the proximal tubule cells at time points of 1 and 2 h, suggesting direct interaction between His-sRAP and megalin expressed in the brush border (Figure 3). At the 6-h time point, His-sRAP disappeared from the proximal tubular cells (Figure 3). Injection of His-sRAP seemed to accelerate the internalization of megalin, which was suggested by the increased dot-like stains with anti-megalin antibody in the cytoplasm (Figure 3, arrows).

Effect of Intraperitoneal Administration of His-sRAP on Subcellular Distribution of Type II Na+/Pi Co-Transporter

Using kidney specimens obtained after a single injection of His-sRAP, we also performed immunostaining for NaPi-II (Figure 3). At the time point of 0 h, NaPi-II was detected in the BBM of proximal tubular cells (Figure 3). Of note, after the intraperitoneal injection, the BBM signal became weaker, and cytoplasmic staining was also observed (Figure 3, arrowheads).

We then performed Western blot analyses using BBM and non-BBM fractions that were obtained from kidneys after a single injection of His-sRAP. When His-probe was used, signals corresponding to His-sRAP were detected in the samples that were obtained 1 and 2 h after the injection in both the BBM and non-BBM fractions (Figure 4). Smaller signals were also detected (data not shown). When the anti-megalin antibody was used, the signal was observed in the BBM fraction at the 0-h time point. At 1 h after the injection, megalin was detected in the non-BBM as well as the BBM fraction, which was consistent with the immunohistochemistry results (Figures 3 and 4). At the 6-h time point, a signal was detected in the BBM fraction, suggesting the recycling of megalin to the apical membrane. For NaPi-II, a signal was detected in the BBM at the time point of 0 h, whereas we observed an intense signal in the non-BBM fraction 1 h after the injection, which revealed the internalization of NaPi-II (Figure 4).

We next investigated whether His-sRAP co-localized with megalin and/or NaPi-II in the process of endocytosis and performed double immunofluorescence staining. When His-probe and the anti-megalin antibody were used, we observed the obvious co-localization of the signals (Figure 5a). However, when His-probe and anti-NaPi-II antibody were used, the signals overlapped only to a small extent in the apical region (Figure 5b).

Increased Urinary Excretion of Low Molecular Weight Proteins, DBP, and Phosphate in Mice Given His-sRAP

Mice were given three consecutive intraperitoneal injections of His-sRAP or a vehicle, and 20-h urine samples were collected before and after the treatment as shown in Figure 1b. In the immunohistochemical examination of kidneys that were obtained 2 h after the third injection, the His-probe detected His-sRAP taken up in the subapical area of proximal tubular cells, as for the case of a single injection of His-sRAP (data not shown). Aliquots of 5 μl of each urine sample were subjected to
SDS-PAGE followed by silver staining. The presence and increased intensity of some signals were observed after treatment with His-sRAP (Figure 6a). Parallel results were obtained when the sample volume was adjusted by creatinine concentration (data not shown). Western blot analysis using the antibody against DBP confirmed the increased urinary loss of DBP on His-sRAP administration (Figure 6b). The urinary concentration of phosphate relative to that of creatinine (u-Pi/Cr) was also elevated after three injections of His-sRAP (Figure 7). We performed immunostaining for NaPi-II using kidneys that were obtained from these animals and found reduced expression in the brush border in the His-sRAP–treated mice compared with that in vehicle-treated mice (Figure 8).

Figure 5. Immunofluorescence of the kidney after the single injection of His-sRAP. Male ICR mice were given a single intraperitoneal injection of recombinant His-sRAP (3.5 mg), and the kidneys were obtained 1 or 2 h after the injection. The kidney specimens were snap-frozen in liquid nitrogen, and 4-μm-thick sections were cut on a cryomicrotome. The sections were incubated with His-probe (raised in goat) and either antimegalin antibody (raised in rabbit) or anti–NaPi-II antibody (raised in rabbit), followed by Alexa Flour 555 donkey anti-goat IgG and Alexa Flour 488 donkey anti-rabbit IgG. (a) The distribution of megalin overlaps with that of His-sRAP in the apical region of the proximal tubular cells. (b) The distribution of NaPi-II partially overlaps with His-sRAP in the apical region.

Decreased Concentration of Serum 25OHD and Unaltered Plasma PTH Levels after His-sRAP Administration

Because increased urinary loss of phosphate was observed after administration of His-sRAP, we determined the concentrations of serum 25OHD and plasma PTH. Consistent with the increased urinary loss of DBP, the concentration of serum 25OHD was significantly decreased by His-sRAP administration (Table 1). Plasma PTH levels were not altered by the treatment with His-sRAP (Table 2).

Discussion

In this study, recombinant soluble RAP lacking the carboxyl-terminal ER retention signal (His-sRAP) proved to be a useful tool to examine the short-term effect of alteration of megalin function when it was administered intraperitoneally to mice. Immunohistochemical examination and Western blot analyses using His-probe clearly demonstrated the uptake of soluble His-sRAP into the proximal tubular cells, suggesting the direct effects of His-sRAP on the functions of these cells.

Figure 6. Increased urinary excretion of low molecular weight proteins, including vitamin D–binding protein (DBP), after intraperitoneal administration of His-sRAP. Mice were given three intraperitoneal injections of His-sRAP or a vehicle, and 20-h urine samples were collected before and after the treatment as described in Materials and Methods. A 5-μl aliquot of each urine sample was subjected to SDS-PAGE followed by silver staining (a) or Western blot analysis using the antibody against DBP (b). R1 to R4, His-sRAP–injected mice; V1, V2, vehicle-injected mice; B, urine samples collected before the treatment; A, urine samples collected after the treatment. The arrow in (a) indicates the signals corresponding to albumin. The asterisks indicate bands with increased intensity after the treatment with His-sRAP.

Figure 7. Increased urinary loss of phosphate after intraperitoneal administration of His-sRAP. Mice were given three intraperitoneal administrations of His-sRAP (○, n = 9) or PBS as a vehicle (○; n = 8), and urine samples were collected before and after the treatment as described in Materials and Methods. Data are expressed as fold changes in the urinary concentration of phosphate relative to that of creatinine (u-Pi/Cr) after the treatment. The circles indicate the values for individual animals.
Figure 8. Decreased amount of NaPi-II protein in the BBM after three administrations of His-sRAP. Kidney specimens were obtained from mice that were given three doses of His-sRAP (3.5 mg/dose, 4-h intervals) or PBS as a vehicle and then were subjected to immunohistochemistry for NaPi-II.

Table 1. Effect of intraperitoneal administration of His-sRAP on serum 25-hydroxyvitamin D

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<th>25OHD (ng/ml)</th>
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<tr>
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<td>Before</td>
</tr>
<tr>
<td>His-sRAP (n=7)</td>
<td>53.4 ± 2.6</td>
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<tr>
<td>Vehicle (n=4)</td>
<td>53.7 ± 1.3</td>
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*Results are shown as mean ± SEM. His-sRAP, histidine (His)-tagged soluble form of receptor-associated protein; 25OHD, 25-hydroxyvitamin D.

*Significantly different from the value before administration (P < 0.01).

Table 2. Effect of intraperitoneal administration of His-sRAP on plasma PTH

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<td>Before</td>
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<tr>
<td>His-sRAP (n=7)</td>
<td>24.4 ± 12.4</td>
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<tr>
<td>Vehicle (n=4)</td>
<td>27.0 ± 5.2</td>
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*Results are shown as mean ± SEM. PTH, parathyroid hormone.

(Figures 3 and 4). In addition, immunostaining using an antibody against megalin demonstrated the accelerated internalization of megalin triggered by the binding of His-sRAP (Figure 3). Western blotting also supported the notion that the injection of His-sRAP caused accelerated megalin-mediated endocytosis (Figure 4). It is interesting that our results suggested that the stimulated megalin function was associated with the internalization of NaPi-II (Figure 3). In addition, urinary excretion of phosphate was increased by sequential administrations of His-sRAP (Figure 7). Phosphaturia observed in our model was not caused by increased secretion of PTH associated with the decrease in serum concentration of 25OHD, as suggested by the unaltered plasma level of PTH after His-sRAP administration. Therefore, we postulated that internalization of megalin, caused by binding of His-sRAP as the ligand, may trigger the removal of NaPi-II from the apical membrane, leading to increased urinary excretion of phosphate. In fact, three sequential administrations of His-sRAP resulted in a decreased amount of NaPi-II in the proximal tubular cells (Figure 8). We assume that the internalized NaPi-II was degraded after the repeated administration of His-sRAP, although the reduction in the amount of NaPi-II protein was not obvious after a single injection. As previously reported, CIC-5–deficient mice exhibit secondary dysfunction of megalin and a defect in trafficking of NaPi-II (17,18). The findings in our model using His-sRAP together with the observation in CIC-5–deficient mice indicate the involvement of megalin in renal phosphate uptake.

In kidney-specific megalin-knockout mice, it was reported that steady-state levels of NaPi-II in the BBM were significantly enhanced and that urinary excretion of phosphate was reduced (14). In addition, systemic administration of PTH resulted in defective retrieval and impaired degradation of NaPi-II in those animals (14). However, in our model, wherein recombinant His-sRAP was administered intraperitoneally, His-sRAP filtered by glomeruli triggered the accelerated internalization of megalin, and internalization and degradation of NaPi-II associated with phosphaturia occurred (Figures 3, 7, and 8). The findings in our model are consistent with those in the study using kidney-specific megalin-knockout mice when the role of NaPi-II in phosphate reabsorption is considered. The difference in NaPi-II distribution and phosphate excretion between the two models originates from the difference in the status of the endocytic machinery. In our model, megalin-mediated endocytosis is accelerated, whereas in the megalin-knockout mouse model, the entire endocytic machinery is impaired. His-sRAP worked as an agonist rather than an antagonist for megalin as suggested by the accelerated megalin-mediated endocytosis, and it is feasible to speculate that there might be some physiologic ligands for megalin that exert similar phosphaturic effects.

The mechanism by which His-sRAP–stimulated megalin-mediated endocytosis caused the internalization of NaPi-II remains unclear at the moment. It has been reported that gentamicin, which is also one of the ligands for megalin, causes endocytosis of NaPi-II (27), suggesting that the effects of His-sRAP are related to the endocytic function of the liganded megalin rather than specific to His-sRAP. Using immunofluorescence, we observed co-localization of His-sRAP with megalin, whereas His-sRAP overlapped with NaPi-II only to a small extent (Figure 5). Previous studies reported the absence of direct interaction between megalin and NaPi-II, and we also failed to detect any direct interaction in an immunoprecipitation study (data not shown). Other molecules might be involved in the alteration of the subcellular distribution of NaPi-II. The mechanism for the internalization of NaPi-II might be distinct from the case of the Na+/H+ exchanger, whereby specific association and co-internalization with megalin have been previously reported in the proximal tubules (28).

Acute impairment of megalin function is often observed in renal tubulopathy caused by aminoglycosides and anticancer drugs (19,20). Hence, the administration of His-sRAP might model these conditions, and our findings suggest that urinary
loss of bioactive proteins, including DBP, may require the supplementation of these substances in such conditions. Our results suggest that acute loss of DBP in urine also may cause a decrease in serum concentration of 25OHD. This finding may suggest that supplementation of 25OHD from the reservoir in the liver is not rapid enough to maintain its serum concentration. The effect of this transient decrease in serum concentration of 25OHD on calcium and bone metabolism is the next issue to be examined.

In conclusion, the intraperitoneal administration of His-sRAP accelerated megalin-mediated endocytosis and caused an alteration of NaPi-II localization and phosphaturia. These results suggest that this experimental system might provide a good model to investigate the relationship between phosphate reabsorption and megalin function.

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References


