PEPT2-CT was observed for PDZ2 and PDZ3, but not for PDZ1 and PDZ4 of PDZK1 (Figure 1c).

In vitro binding of PEPT2 and PDZK1

We used a glutathione-*S*-transferase (GST) pull-down assay to confirm the ability of PEPT2-CT to bind to PDZK1 *in vitro* and validate the protein–protein interaction (Figure 2a). GST fusion proteins bearing the wild-type C-terminus (PEPT2-CT-wt) or C-terminal mutants (PEPT2-CTd3, L279A, and T727A) of PEPT2 were used to pull down *in vitro* translated full-length PDZK1. The data showed the same interaction specificity for PDZK1 and PEPT2 as exhibited in yeast two-hybrid assay (Figure 1a). As expected, the binding of PDZK1 to PEPT2 was completely abolished when the C-terminal PDZ motif was removed (PEPT2-CTd3) or mutated (PEPT2-CT-L729A or PEPT2-CT-T727A) (Figure 2a).

To confirm and quantify the interaction of PEPT2 with PDZK1, we performed surface plasmon resonance experiments using immobilized GST-PEPT2-CT and PDZ2 and PDZ3 of PDZK1 proteins independently fused to maltose-binding protein. As summarized in Table 1, the binding affinities of PDZ2 and PDZ3 of PDZK1 are low ($K_D = 10$ and 15 μM). These values are low in comparison to most PDZ domain interactions ($K_D = 1$ nM–10 μM).[39]

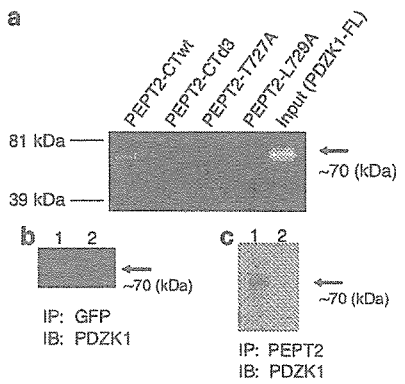

Figure 2 | Interaction of PDZK1 with PEPT2. (a) Full-length PDZK1 polymerase chain reaction product was *in vitro* translated in the presence of Transcend Biotinylated Lysine tRNA (Promega). The *in vitro* translation products were incubated with GST alone (lane 1), GST-PEPT2-CTwt (lane 2), or GST-PEPT2-CTd3 (lane 3) using a ProFound Pull-Down GST Protein:Protein Interaction kit (Pierce). The pull-down products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The input corresponds to the crude *in vitro* translation reaction. Positions of molecular mass standards are indicated on the right. GST fused to PEPT2 C-terminal wt can co-precipitate PDZK1, confirming the specificity found in the yeast two-hybrid system. The mutant form of PEPT2 in which the C-terminal PDZ recognition motif is removed is not able to precipitate PDZK1. (b) Co-immunoprecipitation of PEPT2 and PDZK1 in HEK293 cells. HEK293 cells were transfected with pEGFP-C2 vectors encoding PEPT2-wt (lane 1) or PEPT2-d3 (lane 2) with pcDNA3.1-PDZK1 and then immunoprecipitated with the anti-GFP antibody. Then, the immunoprecipitates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and probed with anti-PDZK1 antibodies. (c) Human kidney membrane fractions were immunoprecipitated with the anti-PEPT2 antibody (lane 1) and control immunoglobulin G (lane 2). The presence of PDZK1 in the immunoprecipitates was determined by Western blotting with the anti-PDZK1 antibody used in a previous study.[29]

Co-immunoprecipitation from heterologous cells and tissue

To demonstrate that PEPT2 and PDZK1 can also interact in mammalian cells, we used a previously prepared rabbit polyclonal antibody against PDZK1.[29] We coexpressed full-length human PEPT2 fused with green fluorescent protein (GFP) (GFP-PEPT2) and PDZK1 in human embryonic kidney (HEK)293 cells. Wild-type GFP-PEPT2 was co-immunoprecipitated with a GFP-specific antibody but GFP-PEPT2 which lacked the last three residues was not precipitated with PDZK1 (Figure 2b).

Furthermore, we demonstrated an association between endogenous PDZK1 and PEPT2 in human tissue by co-immunoprecipitating PEPT2 from human kidney membrane fractions using the anti-PDZK1 antibody, but not control immunoglobulin G (Figure 2c). This result is the evidence that observed interaction occurs between protein partners expressed from endogenous genes in kidneys.

Expression of PEPT2 in human kidney sections

In rats, Pept2 is present at the apical membrane of renal proximal tubules[14,15] and in humans, PDZK1 is reported to be expressed at the apical side of proximal tubular cells.[29,40] To determine whether PEPT2 and PDZK1 colocalize at the apical membrane of renal proximal tubules in humans, we carried out immunostaining of human serial kidney sections using anti-PEPT2 antibody.[41] Consistent with the previous reports, in the renal cortex, PEPT2 immunoreactivities were detected at the apical side of proximal tubular cells (Figure 3).

Table 1 | Characteristics of interaction between PEPT2 C-terminus and PDZK1 PDZ domains 2 and 3 (PDZ2 and PDZ3)

Construct	k_a (1/mM s)	k_d (1/min)	K_D (μM)
PDZK1-PDZ2	7.2×10^2	7.5×10^{-3}	10
PDZK1-PDZ3	3.6×10^2	5.5×10^{-3}	15

The kinetic characteristics of the interaction with immobilized GST-fused PEPT2 C-terminus with the second and third PDZ domains of PDZK1 (PDZ2 and PDZ3) fused with MBP measured by SPR methods are summarized. Association rate constants (k_a), dissociation rate constants (k_d), and equilibrium dissociation constants ($K_D = k_d/k_a$) are given.

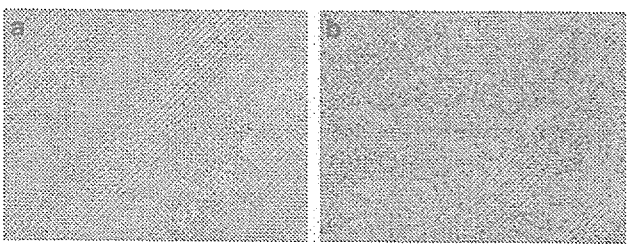

Figure 3 | Immunohistochemical analysis of PEPT2 in human kidney sections. (a and b) Immunohistochemical labeling of PEPT2 by diaminobenzidine reaction of human kidney. (a) PEPT2 was detected in proximal tubules in the cortex. (b) The apical membrane of proximal tubule was immunostained with the anti-hPET2 antibody and no immunostaining was observed in the basolateral membrane and glomeruli. These figures are representative of typical section samples. Original magnifications, (a) × 100 and (b) × 400.

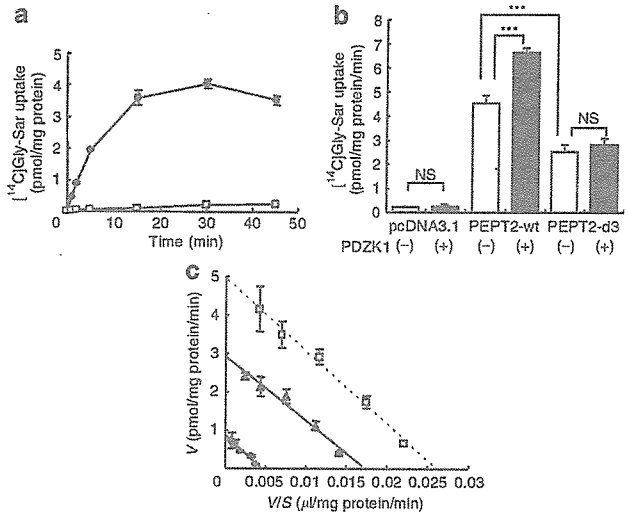

Figure 4 | Effect of PDZK1 on PEPT2-mediated [^{14}C]Gly-Sar transport activity. (a) The time profile of the uptake of [^{14}C]Gly-Sar via PEPT2. Intracellular accumulation of Gly-Sar was linear within 5 min and was significantly greater in PEPT2-wt-transfected HEK293 cells (HEK-PEPT2-wt; filled circles) than that in the mock-transfected cells (HEK-mock; open squares). (b) Coexpression of PEPT2 and PDZK1 increased [^{14}C]Gly-Sar uptake (30 μM) significantly over cells transfected with PEPT2 alone (closed column, middle). This effect was abolished when the C-terminal deletion mutant of PEPT2 was cotransfected with PDZK1 (HEK-PEPT2-d3; closed column, right), confirming that the interaction of PDZK1 with PEPT2 C-terminal domain is responsible for this effect. ***$P<0.001$ (c) Kinetic data using PEPT2-expressing HEK293 cells showed that PDZK1 (open squares) increased the V_{max} from 2.92 to 4.95 fmol/mg protein/min and increased the K_m slightly from 167 to 189 μM, as compared with PEPT2 alone (filled triangles). V_{max} of [^{14}C]Gly-Sar transport via HEK-PEPT2-d3 decreased (0.86 pmol/mg protein/min), whereas its K_m showed no change (187 nM) (filled circles). The kinetic parameters for the uptake via PEPT2 were estimated using $v = V_{max}[S]/(K_m + [S])$, where v is the uptake rate of substrates, $[S]$ is the substrate concentration (μM) in the medium. K_m is the Michaelis–Menten constant (μM) and V_{max} is the maximum uptake rate (pmol/mg of protein/2 min). These parameters were determined using the Eadie–Hofstee equation.

PEPT2 transport activity increases in presence of PDZK1

To determine whether PEPT2 and PDZK1 interaction is required to mediate the increase in PEPT2 transport activity, we transfected HEK293 cells with the pcDNA3.1(+) plasmid containing full-length PEPT2 (HEK-PEPT2-wt), PEPT2 lacking the last three amino acids (HEK-PEPT2-d3), or without an insert (HEK-mock). The time profile of the uptake of [^{14}C]glycylsarcosine (Gly-Sar) via PEPT2 is shown in Figure 4a. Intracellular accumulation of Gly-Sar was linear within 5 min and it was also significantly greater in HEK-PEPT2-wt than that in HEK-mock. After 2 min incubation, we demonstrated that [^{14}C]Gly-Sar uptake via HEK-PEPT2-wt was approximately 20-fold higher than that in HEK-mock and that in HEK-PEPT2-d3 was approximately 12-fold higher than that in HEK mock (Figure 4b). Gly-Sar transport activities significantly increased after PDZK1 coexpression (1.5-fold) (Figure 4b). This effect was not observed when PEPT2-d3 was coexpressed with PDZK1 (Figure 4b).

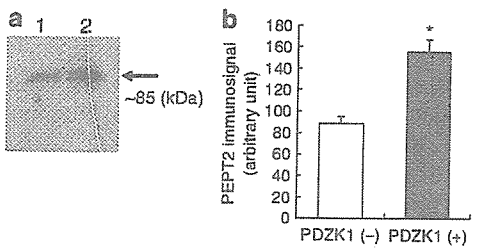

Figure 5 | Surface expression level of PEPT2. (a) cell surface biotinylation analysis of PEPT2 transiently expressing HEK293 cells transfected with vector alone (lane 1), and those transfected with PDZK1 (lane 2). Single bands of approximately 85 kDa, which are consistent with PEPT2, were observed in both lanes. (b) Quantification of immunosignal for PEPT2 ($n=3$, error bars are s.d.). Densitometric analysis was performed using Model DIANA II Imaging System (M&S Instruments Trading Inc., Tokyo, Japan). *$P<0.05$.

Next, we examined the effect of PDZK1 coexpression on the kinetics of [^{14}C]Gly-Sar transport via HEK-PEPT2-wt that had been transfected with pcDNA3.1-PDZK1 or pcDNA3.1 alone. Kinetic data showed that PDZK1 significantly increased V_{max} from 2.92 to 4.95 pmol/mg protein/min and slightly increased K_m from 167 to 189 nM, in comparison to PEPT2 alone (Figure 4c). Interestingly, the V_{max} of [^{14}C]Gly-Sar transport via HEK-PEPT2-d3, decreased (0.86 pmol/mg protein/min), whereas its K_m showed no change (187 nM).

Surface expression level of PEPT2

To determine changes in the cell surface expression level of PEPT2, we used a cell-membrane-impermeant biotinylation reagent to selectively label the cell-surface proteins. After the treatment, the cell lysates from HEK293 cells transfected with PEPT2 and PDZK1 or PEPT2 and mock was collected. The amount of surface-biotinylated PEPT2 expression on plasma membranes increased 1.8-fold (PEPT2 and mock-transfected: 88.3 ± 6.9 vs PEPT2 and PDZK1-transfected: 155.5 ± 11.3 AU, $n=3$) when PDZK1 was coexpressed (Figure 5). This change seems close to the one in V_{max} of PEPT2-mediated transport observed in Figure 4c.

DISCUSSION

The proton-coupled peptide transporter PEPT2 (*SLC15A2*) mediates the high-affinity low-capacity transport of small peptides in the kidney. Therefore, PEPT2 is presumed to contribute to the conservation of peptide-bound amino acids. Although the transport properties and characteristics of substrate recognition for PEPT2 have been well documented, there is less information on PEPT2 regulation. A recent report by Kato *et al.*[31] has provided the novel idea concerning the modulation of PEPT2 function by its associated protein. They demonstrated an interaction between the recombinant PEPT2 C-terminus fused to GST and purified His-tagged PDZK1, but they solely rely on data from *in vitro* binding assays and did not indicate the physiological importance of this interaction. In addition, the yeast two-hybrid screens performed by Gisler *et al.*,[42] using baits

containing single PDZ domains derived from mouse PDZK1, failed to detect Pept2 as a candidate for PDZK1 binding although several membrane proteins including Urat1 were found. To identify PDZK1 as a physiological binding partner of PEPT2, we evaluated this interaction from several viewpoints in this study.

Starting from a yeast two-hybrid screening of a human kidney cDNA library, we have demonstrated PDZK1 to be a physiological interactor of PEPT2. First, we could detect PDZK1 from 64 positive clones by library screening. Second, we could observe the co-immunoprecipitation of PEPT2 and PDZK1 from kidney membrane fractions (Figure 2c). Third, we could demonstrate the localization of PEPT2 protein at the apical side of the renal proximal tubules where PDZK1 is also expressed (Figure 3). These results indicate the physiological meaning of this interaction.

We have further examined this interaction by a yeast two-hybrid assay (Figure 1), an *in vitro* pull-down assay (Figure 2a), co-immunoprecipitation (Figure 2b) and surface plasmon resonance assay (Table 1) of recombinant proteins, as well as by the transport studies (Figure 4) and a cell surface biotinylation assay (Figure 5). These results support the preliminary data presented by Kato *et al.*[31] Moreover, the augmentation of the transport activity by PDZK1 was accompanied by a significant increase in the V_{max} of Gly-Sar transport via PEPT2 (Figure 4c) and was associated with the increased surface expression level of PEPT2 in HEK293 cells (Figure 5). These characteristics are closely similar to those of the URAT1–PDZK1 interaction,[29] and suggest PDZK1 to thus play a similar role in PEPT2–PDZK1 interaction; namely, that PEPT2 is stabilized and/or anchored at the cell membrane, making it less likely to be internalized and subsequently degraded.

Although their functional consequences are the same, there are several differences between the PEPT2–PDZK1 interaction and the URAT1–PDZK1 interaction. First, the frequency of PDZK1 appearing as a positive clones was smaller in the case of PEPT2 (one out of 64) than in the case of URAT1 (35 out of 98). Second, the interaction profiles of PDZK1 ligand against individual PDZ domains of PDZK1 were different, although they have similar C-terminal PDZ motifs: T-K-L for PEPT2 and T-Q-F for URAT1. PEPT2 binds to PDZ2 and PDZ3 (Figure 1), while URAT1 binds to PDZ1, PDZ2, and PDZ4.[29] Third, the binding affinities for each PDZ domain of PDZK1 were more than 10-fold lower for PEPT2 than for URAT1: 10 and 15 μM for PEPT2 (Table 1) and 1.97–514 nM for URAT1.[29] Fourth, when a C-terminal deletion mutant of URAT1 (URAT1-d3) was coexpressed with PDZK1, urate transport activity was not enhanced, but URAT1-d3 still demonstrated a similar urate transport activity to wt URAT1 when expressed without PDZK1. In contrast, the C-terminal deletion mutant of PEPT2 (PEPT2-d3) not only lacked the ability to enhance transport activity when coexpressed with PDZK1, but its transport activity was reduced to half that of the wt PEPT2 (PEPT2-wt) when expressed without PDZK1.

The low frequency of PDZK1 in PEPT2 screening seems consistent with the report of Gisler *et al.*[42] as we mentioned earlier in this paper. In addition to the expression levels of these proteins, the binding affinity is likely to affect the frequency of a particular protein appearing as a positive clone in yeast two-hybrid screening. Therefore, a low frequency of positive clone does not mean that the observed interaction is physiologically less important. Moreover, a low binding affinity may be advantageous for the regulatory dynamics of protein–protein interactions,[27] because a low binding affinity is related to an easier association and dissociation of proteins than a high binding affinity. In particular, PEPT2 has a putative protein kinase C (PKC) recognition site at its C-terminal close to the PDZ motif, whose phosphorylation may interfere with binding to the PDZ domain.[43] It will be interesting to investigate whether the phosphorylation of both PEPT2 and PDZK1 or either protein independently alters the binding affinity of this interaction, in order to clarify the regulatory mechanism for PDZ–ligand interaction.

The decreased transport activity of the PEPT2 C-terminal deletion mutant compared to wt PEPT2, together with the significant reduction in V_{max} (Figure 4) may indicate PDZ motif to thus play another role in the PEPT2-CT: the targeting of the transporter to the plasma membrane. This was originally predicted by Russel *et al.*[28] However, as mentioned above, this phenomenon was not observed in the C-terminal deletion mutant of URAT1 expressed in the same HEK293 cells that have endogenous PDZK1 at low level.[29] Although we frequently detected PDZK1 in the URAT1 screen, we did not find any other binding candidates for URAT1. In the PEPT2 screen, we detected several potential binding partners for PEPT2 besides PDZK1 (manuscript in preparation). It will therefore be important to identify other binding proteins surrounding PEPT2 to understand the potential significance of this interaction.

Recently, PDZ proteins have been recognized as orchestrating scaffolds to achieve concerted functions.[23] PEPT2 mediates an electrogenic proton-coupled cotransport that uses an inward proton gradient to transport small peptides from urine to the cell. Following the concept proposed by Moe, the ability of PDZK1 to couple PEPT2 to the Na^+/H^+ exchanger NHE3 may provide the necessary lumen-to-cell proton gradient, and the multimolecular protein complex will be functionally equivalent to a Na^+/oligopeptide cotransporter. A functional coupling between PEPT2 and NHE1 and/or NHE2 has recently been shown by Wada *et al.*[44] In this paper, we described, for the first time, the exact localization of PEPT2 in the human kidney in addition to its novel regulatory mechanism. PEPT2 proteins are expressed at the apical membrane of renal proximal tubules similarly to rat Pept1 and rat Pept2, which are expressed in the same site.[15] Based on the above findings, human PEPT2 may therefore be involved in the reabsorption of peptides on the apical side of the renal tubules, similar to that of rodent Pept2 and the protein complex surrounding PEPT2 should thus be clarified by identifying other interacting proteins to obtain a

Table 2 | PCR primers used in this study

Construct	Sense primer	Antisense primer
PEPT2-CTwt	5′-CGAATTCCTGCCCGAGACCCAGAG-3′	5′-CTCTCGAGCTAAAACTGTGTGGATTTTA-3′
PEPT2-CTd3	5′-CGAATTCCTGCCCGAGACCCAGAG-3′	5′-CCCTCGAGCTAGGATTTTAGGACAGAGTTC-3′
PEPT2-L727A	5′-CGAATTCCTGCCCGAGACCCAGAG-3′	5′-CCCTCGAGCTAAGCCTGTGTGGATTTTAGGA-3′
PEPT2-T729A	5′-CGAATTCCTGCCCGAGACCCAGAG-3′	5′-CCCTCGAGCTAAAACTGTGCGGATTTTA-3′

PCR, polymerase chain reaction; wt, wild type.

comprehensive understanding of the peptide transport function in the renal proximal tubules.

MATERIALS AND METHODS

Materials

[^{14}C]Glycosylsarcosine (Gly-Sar) (4 Ci/mmol) was obtained from Moravek (Brea, CA, USA). Other materials used included Ham F12 medium from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan), and fetal bovine serum and trypsin from Invitrogen (Carlsbad, CA, USA).

Cloning of human PEPT2 cDNA

The cDNA library was prepared from human kidney poly(-A)$^+$RNA.[45] The 0.46-kb cDNA fragment (24–481 nt of the nucleotide sequence of human PEPT2 (hPEPT2)) was obtained by polymerase chain reaction. This fragment was labeled with [^{32}P]dCTP (T7QuickPrime, Amersham Biosciences, Tokyo, Japan) and used as probe. The screening of the cDNA library was performed as described elsewhere.[46]

Plasmid construction

The C-terminal fragments of wt hPEPT2 cDNA and three mutants (designated d3, L729A, and T727A) were generated by polymerase chain reaction using specific primers (Table 2) and cloned into the *Bam*HI and *Xho*I sites of pEG202 (bait) and pGEX-6P-1 (Amersham Biosciences) to construct PEPT2-CTwt, PEPT2-CTd3, PEPT2-L729A, and PEPT2-T727A. The full-length coding sequences of hPEPT2 (wt) as well as its C-terminal 3-amino-acid-deletion mutant (d3) were inserted into the mammalian expression vector pcDNA3.1 (Invitrogen) for functional analysis and into pEGFP-C2 (Clontech, Tokyo, Japan) for GFP fusion protein preparation. The pcDNA3.1 vector containing the full-length human PDZK1 (hPDZK1) and preys (pJG4–5 and pMAL-C2x) containing single PDZ domains of hPDZK1 were prepared as described previously.[29]

Yeast two-hybrid assay

A human kidney cDNA library was constructed as described previously.[29] A PEPT2 C-terminal bait corresponding to the last 34 amino acids of PEPT2 was used to screen 8.7×10^6 clones of the human kidney cDNA library with the LexA-based GFP two-hybrid system (Grow'n' Glow system; MoBiTec, Göttingen, Germany).

In vitro binding assay

PEPT2-CT for GST fusion protein production in bacteria as reported previously.[47] *In vitro* translation was performed from a plasmid carrying the full-length PDZK1 with the TNT T7 Quick for polymerase chain reaction DNA system (Promega, Tokyo, Japan) in the presence of Transcend Biotinylated tRNA (Promega), as described elsewhere.[29] Of *in vitro*-translated products, (5 μl) was applied into ProFound™ Pull-Down GST Protein:Protein Interaction Kit (Pierce, Rockford, IL, USA) with 50 μl of GST-glutathione-Sepharose resin and protein complexes were eluted according to the manufacturer's instructions.

Surface plasmon resonance

The interactions of PEPT2-CT with the second and third PDZ domains of PDZK1 were investigated using a BIAcore 3000 analytical system (BIAcore AB, Tokyo, Japan). Using an amine coupling kit, GST-fused wt PEPT2-CT or GST alone was attached to a CM5 sensor chip according to the manufacturer's instructions, giving an increase of 11 214 resonance units (RU) for GST-PEPT2-CT or 8,566 resonance units for GST alone. Binding experiments were performed with the PDZK1 single PDZ domains fused with maltose-binding protein as described elsewhere.[29]

Immunohistochemical analysis

We used human single-tissue slides (Biochain, Hayward, CA, USA) for light microscopic immunohistochemical analysis as reported previously.[48] They were treated with 10 μg/ml primary rabbit polyclonal antibodies against PEPT2[41] or PDZK1 (4°C overnight).

Cell culture and transfection

HEK293 cells were maintained in Dulbecco's-modified Eagle's medium supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, penicillin (100 U/ml), and streptomycin (100 mg/ml) (Invitrogen) at 37°C in 5% CO_2. Transient transfection with Lipofectamine 2000 (Invitrogen, Gaithersburg, MD, USA) was performed according to the manufacturer's recommendations.

Immunoprecipitation and immunoblotting

Immunoprecipitation analysis was performed as described previously.[49] Lysates from HEK293 cells that expressed GFP-fused hPEPT2 and hPDZK1 were immunoprecipitated by the anti-GFP antibody (full-length A.v. polyclonal antibody, Clontech). For the co-immunoprecipitation of endogenous PEPT2 and PDZK1, we used human kidney membrane fractions (Biochain) and added the anti-PEPT2 antibody or control immunoglobulin G to this solution. After overnight incubation, PEPT2 and PDZK1 were immunoprecipitated using the Seize Classic (A) Immunoprecipitation kit (Pierce). The affinity-purified rabbit PDZK1 antibody and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Amersham Biosciences) were used for immunoblotting with enhanced chemiluminescence reagents (ECL Plus, Amersham Biosciences).

Gly-Sar transport activity assay

HEK293 cells were plated on 24-well culture plates at a density of 2×10^5 cells/well 24 h prior to transfection, and they were transfected as described above. After 36 h, the culture medium was removed, and the cells were washed three times and incubated in

serum-free Hank's solution (containing in mM: 125 NaCl, 5.6 glucose, 4.8 KCl, 1. 2 $MgSO_4 \cdot 7H_2O$, 1.2 KH_2PO_4, 1.3 $CaCl_2 \cdot 2H_2O$, 25 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 6.0)) for 10 min. The uptake study was started by adding 500 μl of solution containing 30 μM [^{14}C]Gly-Sar to the plate. After 2 min, the cells were washed twice in an ice-cold solution, and lysed in 0.1 N NaOH for 20 min for scintillation counting.

To determine the kinetic parameters, the concentrations of Gly-Sar were varied from 30 to 1000 μM. PEPT2-mediated Gly-Sar uptake was calculated as the difference between the uptake rates into HEK293 cells transiently expressing PEPT2 and those into HEK293 cells transfected with the vector (pcDNA3.1, Invitrogen) only.

Cell surface biotinylation

Surface biotinylation of PEPT2 at the plasma membrane was performed as described elsewhere.[49] Surface proteins in HEK293 cells transfected with pcDNA3.1(+)-hPEPT2 and pcDNA3.1(+)-hPDZK1 or pcDNA3.1(+) empty vector (mock) were biotinylated with Sulfo-NHS-SS-Biotin (Pierce) (0.5 mg/ml) in phosphate-buffered saline for 30 min at 4°C. Cell lysates were then incubated with Ultralink-immobilized NeutrAvidin beads (Pierce) to precipitate biotinylated proteins. PEPT2 was detected with polyclonal PEPT2 antibody (1:10,000).[41]

Statistical analysis

Uptake experiments were conducted three times, and each uptake experiment was performed in triplicate. Values are presented as the mean±s.e. Statistical significance was determined by Student's *t*-test.

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Molecular physiology of renal organic anion transporters

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Invited Review

Molecular physiology of renal organic anion transporters

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[1]Department of Pediatrics, Faculty of Medicine, The University of Tokyo, [2]Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo; [3]Department of Nephrology, Graduate School of Medical Sciences, Kumamoto Univiversity, Kumamoto; and [4]Fuji Biomedix Company, Tokyo, Japan

Sekine, Takashi, Hiroki Miyazaki, and Hitoshi Endou. Molecular physiology of renal organic anion transporters. *Am J Physiol Renal Physiol* 290: F251–F261, 2006; doi:10.1152/ajprenal.00439.2004.—Recent advances in molecular biology have identified three organic anion transporter families: the organic anion transporter (OAT) family encoded by *SLC22A*, the organic anion transporting peptide (OATP) family encoded by *SLC21A* (*SLCO*), and the multidrug resistance-associated protein (MRP) family encoded by *ABCC*. These families play critical roles in the transepithelial transport of organic anions in the kidneys as well as in other tissues such as the liver and brain. Among these families, the OAT family plays the central role in renal organic anion transport. Knowledge of these three families at the molecular level, such as substrate selectivity, tissue distribution, and gene localization, is rapidly increasing. In this review, we will give an overview of molecular information on renal organic anion transporters and describe recent topics such as the regulatory mechanisms and molecular physiology of urate transport. We will also discuss the physiological roles of each organic anion transporter in the light of the transepithelial transport of organic anions in the kidneys.

OAT; urate; organic anion transporting peptide; multidrug resistance-associated protein

THE ORGANIC ANION (OA) transport system has been a major subject in renal physiology over the past 100 years, because this system represents the tubular secretory pathway. OAs include numerous substances of both endogenous and exogenous origins, and the renal OA transport system plays a pivotal role in the elimination of potentially toxic compounds including metabolites, xenobiotics, and drugs. Because of its complexity, there are still limitations in a detailed analysis of the renal OA transport system by physiological techniques. A notable advance was made in the 1990s by the identification of three major organic anion transporter families: the OAT (organic anion transporter) family encoded by *SLC22A*, the organic anion transporting peptide (OATP) family encoded by *SLCO*, and the multidrug resistance-associated protein (MRP) family encoded by *ABCC* (ATP-binding cassette) superfamily. The identification of each transporter enabled us to perform precise functional analyses of the system. Molecular knowledge in this area has rapidly increased, and various information has appeared within the last few years. In this review, first, we will give an overview of molecular information on OA transporters. Then, we will focus on recent advances, particularly those in the study of the OAT family, which represents the major and classic renal OA transport system. With regard to the OATP and MRP families, we will touch only potentially important points in renal physiology. The major topics described in this paper are as follows: *1*) regulatory mechanisms of OA transporters, such as gender differences, intracellular signaling, genomic organization, pathophysiological states and scaffolding proteines; *2*) molecular physiology of the renal urate transporter; and *3*) organization of transepithelial transport of organic anions. For historical and physiological backgrounds on renal OA transport systems, excellent reviews (50, 52, 71) are recommended. Extensive reviews on recent molecular information on the OA transporter family are also available (8, 77).

OVERVIEW OF OA TRANSPORTER FAMILIES

In the body, the kidney as well as the liver are equipped with excretory systems for OAs. Roughly speaking, previous physiological and pharmacological knowledge indicated a rule for the route of OA elimination. Relatively small (molecular weight <400 ~ 500 kDa) and hydrophilic OAs, such as PAH, are mainly excreted via the kidneys. OA with these characteristics are classified as type I OA (77). Conversely, relatively large (molecular weight >400 ~ 500 kDa) and hydrophobic OAs, such as bile acids and glucronide conjugates, are preferentially excreted by the liver. These OAs are classified as type II (77). This general rule for the OA elimination pathway is now explained in molecular terms. Type I OAs are preferable substrates of members of the OAT family, which are predominantly expressed in the kidneys. Thus, in this review, we will mainly describe the OAT family. Figure 1 shows organic anion transporters such as OATs, OATPs, and MRPs in the renal proximal tubule.

Essential information on the OAT members is described in Table 1. For the OATP and MRP families, major findings are summarized in Tables 2 and 3, respectively, and only issues concerning renal physiology are discussed to integrate their information into transepithelial transport of OA.

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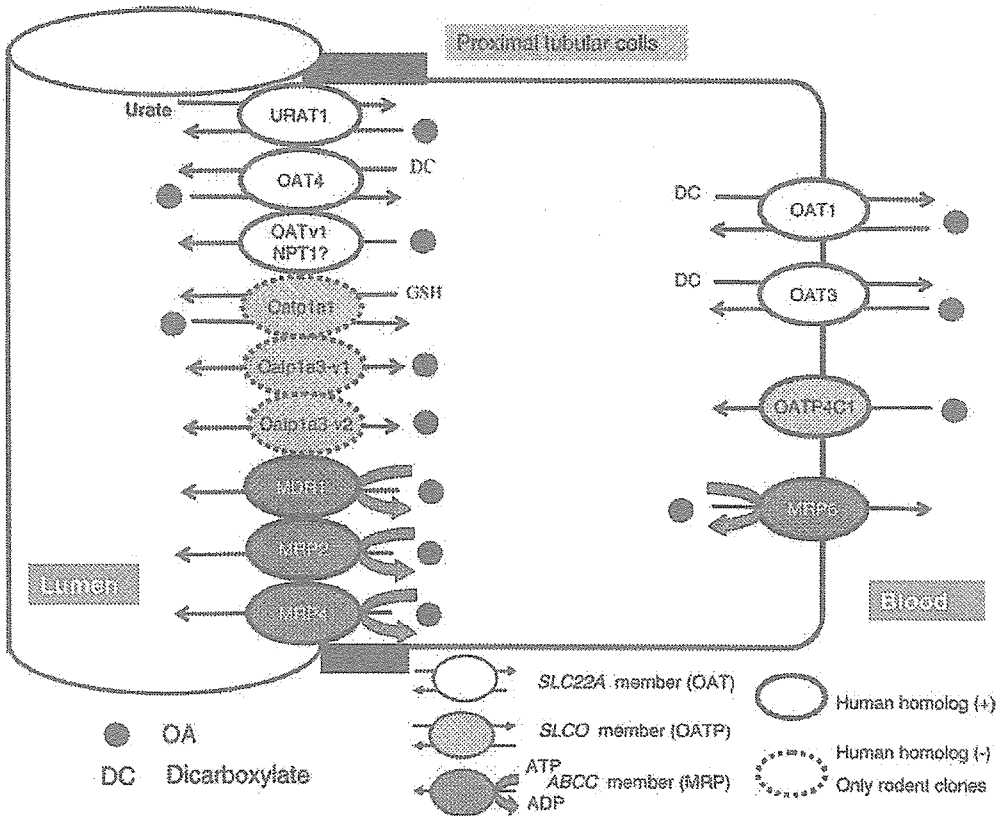

Fig. 1. Organic anion (OA) transporters in proximal tubular cells. White ovals, *SLC22A* members; light grey ovals, *SLCO* members; dark grey ovals, multidrug resistance-associated proteins (MRPs; *ABCC* members). In the basolateral membrane, OAT1 and OAT3 mediate uptake of a wide range of relatively small and hydrophilic OAs from plasma. OATP4C1 is shown to transport digoxin. In the apical membrane, many OA transporters are identified. The role of URAT1 as an efflux transporter for various OAs into tubular lumen is suggested. In regard to the OATP members, large species differences are noted and their contribution to transepithelial transport of OA is still unclear. Oatp1a3v1 and Oatp1a3v2 could participate in tubular reabsorption and/or secretion of relatively hydrophobic anions such as bile acids, methotrexate, and PGE_2. MRP2 and MRP4 extrude type II OAs from the cell into tubular lumen. MRP4 is shown to mediate the transport of PAH. OATv1 and its putative human ortholog NPT1 belong to the distinct transporter family (SLC17A). OATv1 would function as a voltage-driven OA transporter, which mediates efflux of OAs. Transporters whose human ortholog is not identified are depicted by dotted lines.

The OAT Family (SLC22A)

The prototypical member of this family, OAT1, was identified in 1997 as a PAH transporter by functional cloning (57, 64, 76) and revealed to be the rat (57, 64) and flounder (76) ortholog of a previously identified mouse transporter protein with an unknown function (43), respectively. Thus far, six isoforms of OAT have been identified (Table 1). OAT members are structurally similar to organic cation transporters (OCTs) (35); both belong to the *SLC22A* gene family.

OAT1 is expressed at the basolateral membrane of proximal tubular cells and functions as an organic anion/dicarboxylate exchanger that takes up OA from the plasma into proximal tubular cells. In the kidneys, OAT1 expression is restricted to proximal tubular cells, in particular the S2 segment (36). OAT1 interacts with >100 compounds, and its substrates include endogenous substances, such as dicarboxylates, cyclic nucleotides, prostaglandins, and urate as well as exogenous ones, such as drugs and environmental compounds (59). Species differences and gender differences are demonstrated for OAT1 expression.

OAT2, originally isolated from a mouse liver as a "novel liver-specific transporter" (NLT) of unknown function, was revealed to be an OAT (58). OAT2 is expressed in the liver and kidneys. Its intrarenal localization is still controversial, and its mode of transport is unknown. Typical substrates of OAT2 are salicylate, acetylsalicylate, PGE_2, dicarboxylates, and PAH. Marked gender differences in OAT2 expression are observed (33).

OAT3 is expressed in the kidneys, brain, eyes, and liver (38). In the kidneys, OAT3 is localized at the basolateral membrane of the proximal tubular cells. In the brain, OAT3 is localized to the apical membrane of the choroid plexus (65). OAT3 exhibits a wide substrate selectivity similar to OAT1. OAT3 mediates the high-affinity transport of estrone sulfate, dicarboxylates, ochratoxin A, PAH, and various drugs, even including the cationic drug cimetidine. OAT3 has been identified as an OA/dicarboxylate exchanger similar to OAT1 (4, 66). Species differences and gender differences are noted for OAT3.

OAT4 was cloned from human kidneys (11). OAT4 mRNA is abundantly expressed in the kidneys and placenta. So far, the OAT4 orthologs in rodents and other species have not been identified. OAT4 is localized at the apical membrane of proximal tubules (2). In the placenta, OAT4 is expressed on the

Table 1. *Members of the OAT family*

Gene Product (Gene Symbol)	Identified Species	Gender Difference	Tissue Distribution	Transport Mechanism	Membrane Localization in PT	Representative Substrates
OAT1 (*SLC22A6*)	*Caenorhabditis elegans*, flounder, human, mouse, pig, rabbit, rat	Yes (rat) Male > female	Brain, kidneys, placenta, eyes, smooth muscle	OA/DC exchanger	Basolateral	PAH, DC, PGs, cyclic nucleotides, urate, folate, diuretics, ACE inhibitors, antiviral agents, β-lactam antibiotics, antineoplastics, mycotoxins, sulfate conjugates, glucuronide conjugates, cystein conjugates, ochratoxin A, NSAIDs, uremic toxins
OAT2 (*SLC22A7*)	Human, mouse, rat	Yes (mouse)*	Kidneys, liver	Unknown	†	PAH, DC, PGE_2, salicylate, MTX, acetylsalicylate
OAT3 (*SLC22A8*)	Human, rabbit, mouse, pig, rat	Yes (rat) Male > female	Bone, brain, eyes, kidneys, liver, adrenal glands	OA/DC exchanger	Basolateral	ES, DHEA-S, PAH, DC, urate, cyclic nucleotides, cortisol, cimetidine, salicylate, uremic toxins, MTX, β-lactam antibiotics, ochratoxin A,
OAT4 (*SLC22A11*)	Human	Unknown	Kidneys, placenta	OA/DC exchanger	Apical	ES, DHEA-S, PAH, ochratoxin A, PGE_2, $PGF_{2\alpha}$
URAT1 (*SLC22A12*)	Human, mouse	Yes (mouse) Male > female	Brain, kidneys	Urate/anion exchanger	Apical	Urate
OAT5 (*SLC22A19*)	Mouse, rat	‡	Kidneys	Unknown	Unknown	Ochratoxin A

OA, organic anion; OAT, OA transporter; PAH, *para*-aminohippurate; DC, dicarboxylates; ES, estrone sulfate; DHEA-S, dehydroepiandrosterone sulfate; MTX, methotrexate; NSAIDs, nonsteroidal inflammatory drugs; PG, prostaglandin; PT, proximal tubular cell; ACE, angiotensin-converting enzyme. *Expression level of OAT2 shows gender differences in the liver (female > male), whereas no significant difference are observed in the kidneys. †Membrane and intrarenal localizations of OAT2 are still controversial. ‡OAT5 is expressed in both males and females, but quantitative differences are still unexplored.

fetal side of the syncytiotrophoblast (70). OAT4 mediates the high-affinity transport of estrone sulfate, dehydroepiandrosterone sulfate, ochratoxin A, and PGE_2 and PGF (32). Recently, OAT4 has been explored to be an OA/dicarboxylate exchanger (13).

OAT5 has identified from mice (79). The mRNA of OAT5 is highly expressed in the kidneys of adult mice and rats. Mouse OAT5 is encoded by *Slc22a19*, which is localized to chromosome 19 near those for OAT1 and OAT3. OAT5 mediates the transport of ochratoxin A (K_m: 2.0 μM). OAT5 shows neither *cis*-inhibition nor *trans*-stimulation by glutarate, unlike OAT1 and OAT3, and it is unaffected by short-circuiting of membrane potential. Gender differences of OAT5 expressions remain to be elucidated.

URAT1 is a urate/anion exchanger (14). URAT1 will be described below in (further) detail (Fig. 2).

Recently, a putative OA transporter, namely OAT6, was isolated (51). OAT6 is expressed predominantly in the mouse olfactory mucosa but not in the kidneys or brain. OAT6 expression is also observed in the testes. The genomic localization of OAT6 is proximal to the OAT1/OAT3 gene pair. Based on sequence homologies, this protein was designated OAT6. However, further studies are necessary in identifying its substrate specificity and therefore its involvement in the transport of OAs.

Table 2. *Members of the OATP family expressed in the kidney*

Gene Product (Gene Symbol)	Old Name	Identified Species	Tissue Distribution	Intrarenal and Membrane Localization	Representative Substrates
Oatp1a1 (*Slco1a1*)	Oatp1	Mouse, rat	Brain, colon, kidneys, liver, lungs, small intestine	PT (S3): apical	BSP, taurocholate, $E_2$17βG, LTC_4, DNP-SG, T3, T4, aldosterone, cortisol, ouabain, ochratoxin A, temocrapirat, enalapril
OATP1A2 (*SLCO1A2*)	OATP-A	Human	Brain, kidneys, liver	CCD: basolateral	BSP, cholate, taurocholate, DHEA-S, E_2 17βG, PGE_2, T3, T4, chlorambucil, fexofenadine, ouabain, BQ123, CRC220, ochratoxin A
Oatp1a3 v1 (*Slco1a3*)	Oat-K1	Rat	Kidneys	PT (S3): apical	Taurochorate, E_2 17βG, ES, DHES, folate, T3, T4, MTX
Oatp1a3 v2 (*Slco1a3*)	Oat-K2	Rat	Kidneys	PT + CCD: apical	Taurochorate, E_2 17βG, ES, DHES, folate, T3, T4, MTX
Oatp1a5 (*Slco1a7*)	Oatp3	Mouse, rat	Kidneys, lungs, retina	Unknown	Taurochorate, T3, T4
OATP2A1 (*SLCO2A1*)	PGT	Human, rat	Ubiquitous	Unknown	PGs
OATP2B1 (*SLCO2B1*)	OATP-B	Human	Brain, heart, intestine, kidneys, liver, placenta	Unknown	BSP, ES, DHEA-S, PC-G
OATP3A1 (*SLCO3A1*)	OATP-D	Human, mouse	Ubiquitous	Unknown	ES, PGE_2, PC-G
OATP4A1 (*SLCO4A1*)	OATP-E	Human, mouse, rat	Ubiquitous	Unknown	Taurocholate, E_2 17βG, ES, PGE_2, T3, T4, PC-G
OATP4C1 (*SLCO4C1*)	OATP-H	Human, rat	Kidneys	PT: basolateral	T3, digoxin, ouabine
Oatp1a6 (*Slco1a6*)	Oatp5	Mouse, rat	Kidneys	Unknown	Unknown

OATP, OA-transporting peptide; BSP, bromosulfophthalein; $E_2$17βG, estradiol 17β-D-glucuronide; LTC_4, leukotrien C_4; DNP-SG, *S*-(dinitrophenyl)-glutathione; PC-G, benzylpenicillin; CCD, cortical collecting duct.

Table 3. *Members of the MRP family expressed in the kidney*

Gene Product (Gene Symbol)	Identified Species	Tissue Distribution	Intrarenal and Membrane Localization	Representative Substrates
MDR1 (*ABCB1*)	Human, mouse, rat	Brain, intestine, kidneys	PT: apical	ES, DHEA-S, E_2 17βG, rhodamine123, anti-HIV agents, anticancer agents, verapamil, fluo 3, calcein, cardiac glycosides
MRP1 (*ABCC1*)	Human, mouse	Ubiquitous	MTAL~CCD: basolateral	LTC_4, DNP-SG, E_2 17βG, GSSG, AFB_1-SG, PGA_1-SG, PGA_2-SG, GSH, PAH, MTX, etoposide-*G*, fluo 3, calcein
MRP2 (*ABCC2*)	Human, mouse, rat	Brain, intestine, kidneys, liver	PT: apical	LTC_4, DNP-SG, E_2 17βG, folate, glycholate, urate, cAMP, cGMP, PMEA, AZTMP, MTX, PAH, etoposide-G, fluo 3, calcein
MRP3 (*ABCC3*)	Human, rat	Adrenals, intestine, kidneys, liver, pancreas	CCD: basolateral	LTC_4, DNP-SG, E_2 17βG, folate, glycholate, MTX
MRP4 (*ABCC4*)	Human, rat.	Ubiquitous	PT: apical	E_2 17βG, urate, cAMP, cGMP, PMEA, AZTMP, MTX, adefovir.
MRP5 (*ABCC5*)	Human	Ubiquitous	Unknown: basolateral	GSH, cAMP, cGMP, 6-MP, adefovir, DNP-SG, CMFDA. BCECF, FDA
MRP6 (*ABCC6*)	Human	Kidneys, liver	PT: basolateral	LTC_4, NEM-SG, BQ123

MRP, multidrug resistance-associated protein; AFB_1-SG, S-(aflatoxin B_1)-glutathione; AZTMP, azidothymidine monophosphate; CMFDA, 5-chlomethyl-fluorescein; 6-MP, 6-mercaptopurine; MTAL, medullary thick ascending limb; NEM-SG, *N*-ethylmaleimide glutathione; PMEA, 9-(2-phosphonylmethoxyethyl)-adenine; BQ123 is an endothelin-receptor antagonist, cyclo[Trp-Asp-Pro-Val-Leu].

The OATP family (SLCO)

The first member of this family, oatp1, was identified from rat liver by an expression cloning method as a sodium-independent bile acid transporter (29). Thus far, 11 human isoforms and 14 rat isoforms have been identified in the OATP family (45, 47). Although some OATPs are selectively involved in the hepatic uptake of bulky and relatively hydrophobic OAs, most OATPs are expressed in many tissues, such as the blood-brain barrier, choroids plexus, lungs, heart, intestine, kidneys, placenta, and testes (22). To clarify the confusing and species-dependent "old" nomenclature, a novel nomenclature has recently been assigned to the OATP family (Table 2). The OATP superfamily was subdivided into several families (≧40% amino acid sequence identity) and subfamilies (≧60% amino acid sequence identity) (22). The OATP family is now divided into six families (OATP1–OATP6). There are considerable species differences in the OATP family among rodents and humans. Among human OATPs, only OATP4C1 is mainly expressed in the kidneys. Oatp1a3v1 (previous name: OAT-K1) and Oatp1a3v2 (previous name: OAT-K2) are specifically

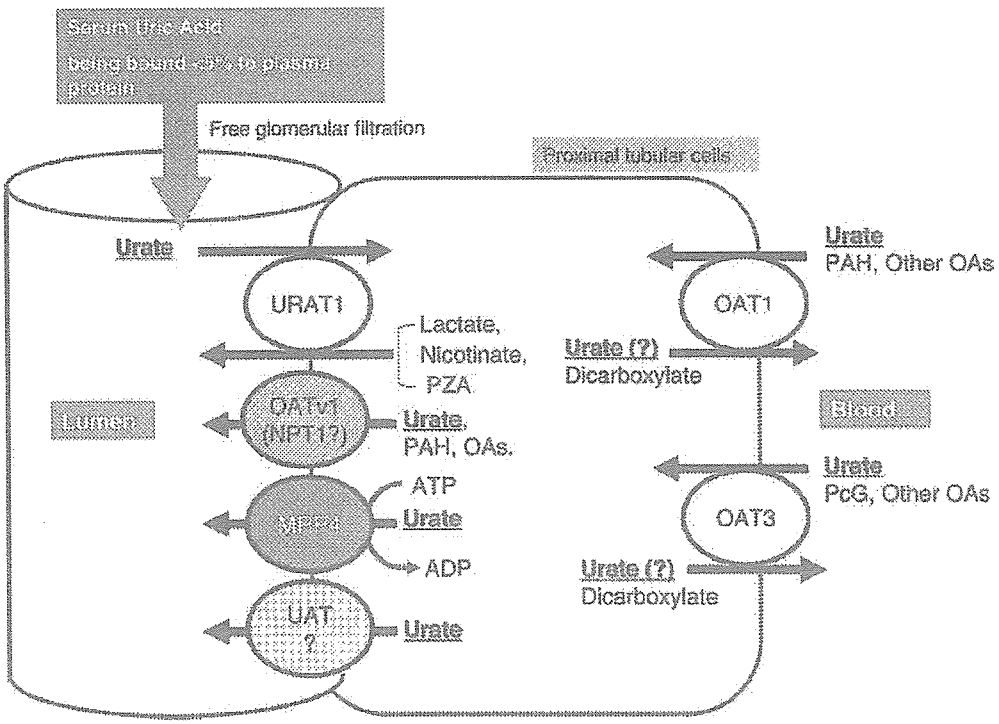

Fig. 2. Proposed model of transcellular urate transport in the proximal tubular cells. To date, 6 membrane proteins, namely, URAT1, OATv1, OAT1, OAT3, MRP4, and UAT, have been identified as urate transporters. URAT1 is an apical urate/anion exchanger responsible for reabsorption of urate from glomerular filtrate. OATv1 is a voltage-driven OA transporter, and it also mediates the transport of urate. UAT is supposed to be an electrogenic urate channel. Urate is an endogenous substrate for OAT1 and OAT3. OAT1 and/or OAT3 presumably play a role in uptake of urate from peritubular plasma. A comprehensive understanding of the renal handling of urate remains to be elucidated.

expressed in the rat. Oatp1a1 (previous name: oatp1), Oatp1a5 (previous name: oatp3), Oatp1a6 (previous name: oatp5), and Oatp4c1 are expressed in rodent kidneys. The orthologs of these isoforms, except OATP4C1, are absent in humans. Because of the above-mentioned remarkable species differences in OATP, it is difficult to assign distinct physiological roles to each OATP in the kidneys. The role of OATP4C1 in the kidneys is evident. There are several important substances that are preferable substrates for the OATP family, which are mainly excreted via the kidneys. One example is digoxin, a cardiac glycoside. The exit pathway for digoxin at the apical membrane of proximal tubular cells has been assumed to be an ATP-dependent efflux pump, P-glycoprotein (P-gp). However, the basolateral entrance for digoxin was as yet unknown. Recently, OATP4C1, has been revealed to be a digoxin transporter (46). OATP4C1 is expressed exclusively in the basolateral membrane of proximal tubular cells and mediates the high-affinity transport of digoxin (K_m: 7.8 μM) and ouabain (K_m: 0.38 μM), as well as thyroid hormones such as triiodothyronine (K_m: 5.9 μM). These data suggest that OATP4C1 is a digoxin transporter localized in the basolateral membrane of proximal tubular cells and plays a central role in the renal elimination of digoxin.

The MRP Family (ABCC Gene Family)

The MRP family consists of primarily active transporter with ATP-binding cassette motifs. The prototype of this family is P-gp, which extrudes various hydrophobic molecules, particularly antineoplastic compounds, such as vincristine, vinblastine, adriamycin, and daunorubicin, and confers multidrug resistance on cancer cells (20).

MRP1 and MRP2 were isolated from cancer cells with multidrug resistance that do not express P-gp. In addition to antineoplastic drugs, MRP2 transports glucronides and cysteine conjugates, and it is expressed in the canalicular membrane of hepatocytes (55). MRP2-deficient mice lack the activity to extrude conjugate anions from the liver, resulting in the phenotype of the Dubin-Johnson syndrome (31). Thus far, many isoforms have been identified in the MRP family (40, 55), and several of these isoforms are expressed in the apical membrane of proximal tubular cells (Table 3 and Fig. 1). MRP members in proximal tubular cells supposedly function as an extrusion pump for OAs from the apical membrane, especially type II OA. With respect to renal physiology and pharmacology, particular attention should be paid to two isoforms, namely, MRP2 and MRP4. MRP2 has been shown to transport PAH, but its affinity for PAH is low (K_m: 2 mM). The observation that the renal excretion of PAH in isolated perfused kidneys from Mrp2-deficient rats is not significantly different from those in the kidneys from wild-type rats suggests a modest, if any, contribution of MRP2 to the efflux of PAH (62). In contrast, human MRP4, which is also localized in the apical membrane of proximal tubular cells, transports PAH with a much higher affinity (K_m: 160 μM) compared with MRP2. Furthermore, real-time PCR and Western blot analysis showed that the renal cortical expression of MRP4 is approximately fivefold higher than that of MRP2 (62). These data demonstrate that MRP4 plays a certain role in the efflux of PAH and several type I OAs, such as urate, cAMP, and cGMP into the tubular lumen (74).

REGULATORY MECHANISMS IN THE OAT FAMILY

Gender Differences and Regulation by Sex Hormones

Kobayashi et al. (33) demonstrated gender differences in OAT2 expressions in mice. The OAT2 mRNA expression level in the liver is higher than that in the kidneys of male mice, whereas it is equivalent between the liver and the kidneys in female mice. The expression level of OAT2 in castrated male mice kidneys is markedly increased, but it is decreased by testosterone. Buist et al. (6, 7) reported similar gender differences in OAT1, OAT2, and OAT3 in the mice and rat. The mRNA expression level of OAT1 in the kidney and that of OAT3 in the liver are higher in male rats than in female rats. In contrast, the OAT2 expression level in the liver is higher in female rats than in male rats. In male rats, hypophysectomy (HX) decreases the OAT1, OAT2, and OAT3 expression levels. In female rats, HX decreases mRNA level of OAT2, but increases OAT3 expression level. Similar results were also observed in mice. Gender differences in OAT expression have been confirmed at the protein level. The OAT1 expression in female rat kidneys is only 40% that in the male rat kidneys (10). OAT1 and OAT3 levels in the renal cortex are higher in male rats than those in female rats. These differences are only observed in adult rats, not in prepubertal rats. These differences are enhanced by androgens and inhibited by estrogens (42). Gender differences are also observed in the urate transporter URAT1. The expression of mouse URAT1 is higher in male mice than that in female mice. This point will be discussed below.

These results showed gender differences and the sex hormone regulation of OAT expression in the kidneys as well as in other tissues. This fact is important in the following context. First, the gender differences imply the existence of potentially important sex-related endogenous substrates of OATs, such as the sulfate conjugates of steroid hormones. If OATs serve only as a secretory pathway for xenobiotics, the gender differences are difficult to explain. OATs might function primarily as transporters for endogenous substrates associated with sex hormones. Second, the gender difference in OAT expression would directly influence pharmacokinetics and toxicokinetics. The administration of potentially toxic drugs whose elimination depends on tubular secretion, such as methotrexate, should be well designed. The effect of estrogens on OAT expression is important clinically, because the estrogen level decreases rapidly in elderly females.

Intracellular Signaling

Studies using perfused proximal tubules, opossum kidney cells and the S2 segments of single, nonperfused rabbit proximal tubules have demonstrated the regulation of OA transport by PKC (68). The uptake or transepithelial transport of OA was inhibited after exposure to PMA, an activator of PKC, and this inhibitory effect of PMA was rescued by pretreatment with an inhibitor of PKC. There are several PKC phospholylation sites in the intracellular loops in OATs (48), and their possible regulation has been examined. The downregulation of OA transport by PKC activation was demonstrated in rats (72) and human (44) and mouse OAT1 (78). The same direction of PKC regulation was also observed in rat (67) and rabbit (63) (rb)OAT3. Takeda et al. (67) demonstrated that PMA attenuated the cellular uptake of estrone sulfate (ES) in OAT3-

expressing cells in a dose- and time-dependent manner. PMA treatment decreased V_{max} but not the K_m for ES transport by OAT3-expressing cells, suggesting that this downregulation may be due to the inhibition of the translocation or internalization of rOAT3. You et al. (78) demonstrated that the downregulation of PAH transport via PKC activation occurs without the direct phospholylation of mouse OAT1 (for a more extensive overview on this matter, see Ref. 68).

Genomic Organization and Possible Transcriptional Regulation

Eraly et al. (16) investigated regulatory elements using comparative genomics approaches. Binding sites for transcription factors, including PAX1, PBX, WT1, and HNF1, are present within the evolutionarily conserved noncoding sequences of OATs, although the roles of these transcription factors on expression of OATs have not been clarified. Genes encoding OATs are located in the human and mouse genomes as tightly linked pairs; OAT1 and OAT3, hUST3 and hOAT5, and OAT4 and URAT1/renal-specific transporter (RST). The exception is OAT2, for which no paired member exists and that is located far from all other OATs on choromoseme 6p21. These linked gene pairs are also close phylogenetically. Pair members exhibit similar tissue distributions; the coregulation of the genes within each pair might occur (16). Genomic organization of OATs with respect to their regulation is an important issue to be solved in further studies. Eraly et al. also investigated the molecular phylogeny of the *SLC22A* families. Several OATs in *Drosophila* and *Caenorhabditis elegans* are developmentally regulated. The analysis of intron phasing suggests that the OAT, OCT, and OCTN lineages of the slc22 family formed after the divergence of vertebrates and invertebrates. Subsequently, these lineages expanded through independent tandem duplications to produce multiple gene pairs (17, 18). For human OAT1, four splice variants, i.e., OAT1–1 to OAT1–4, exist, whereas individual roles of these variants are not known (3, 23).

Pathophysiological States

Recent studies indicated that the expressions of OATs are affected in pathophysiological states. During the progression of renal insufficiency, various uremic toxins derived from dietary proteins accumulate in uremic plasma. Many uremic toxins are OA; their accumulation in the kidney is a result of renal dysfunction, and this also accelerates underlying renal diseases. Enomoto et al. (15) demonstrated that the administration of indoxyl sulfate (IS) into 5/6 nephrectomized rats enhanced the progression of chronic renal failure. Immunohistochemical studies revealed that the amount of IS in proximal tubular cells and the expression of OAT1 and OAT3 are both enhanced in the chronic renal failure model. Deguchi et al. (12) analyzed the transport of various uremic toxins, such as IS, 3-carboxy-4-methyl-5-propyl-2-furanpropionate, indole acetate, and hippurate via OAT1 and OAT3. They showed that rat Oat1/human OAT1, and rat Oat3/human OAT3 play major roles in the renal uptake of these uremic toxins. The enhanced expression of OATs in uremic circumstances indicates compensatory effects for elimination of uremic substrances and leads to the progress of the underlying diseases by accumulating harmful uremic toxins in proximal tubular cells. The expressions of OATs are affected by several renal dysfunction models. Bilateral urethral obstruction in adult male rats increases OAT1 expression level (75). Acute arterial calcinosis induced by bolus injection of vitamin D_3 also increases the OAT1 expression level (53). In various human kidney diseases, OAT expression was analyzed using a real-time PCR method (56). The data indicate that OAT3 expression is decreased in patients with renal diseases. OAT expression is influenced not only by renal diseases but also by hepatic diseases. A compensatory enhancement of OA excretion in rats with acute biliary obstruction was demonstrated for OAT1 (5). The significance and relevance of a change in OAT expression in the pathophysiological state are an important issue for future studies.

Scaffolding Protein

It has been demonstrated that several transporters located in the apical membrane of proximal tubular cells possess a PDZ motif at their COOH-terminal end (55). The PDZ motif is a protein-protein interaction module and is composed of three amino acid residues: S/T-X-Φ (where X is any amino acid, and Φ is a hydrophobic residue). PDZ motifs interact with PDZ proteins via a PDZ domain consisting of 80–90 amino acids. To date, four PDZ proteins are identified in the apical membrane of renal proximal tubular cells, and PDZK1 and Na^+/H^+ exchanger regulatory factor (NHERF)1 has been shown to interact with organic anion transporters such as URAT1, OAT4 (1), Na^+-dependent phosphate cotransporter type 1 (NPT1) (19), MRP2 (34), and MRP4 (55). The coexpression of URAT1 and PDZK1 in HEK293 cells increased URAT1 transport activity. This result indicates that the PDZ protein affects the transport activity of OATs and regulates the transepithelial transport of OAs (1). Importantly, recent studies revealed that PDZK1 and NHERF1 could interact (19). The ability of PDZK1 and NHERF1 to bind to several apical transporters simultaneously, together with the fact that PDZK1 and NHERF1, respectively, form homo- (34, 60) and heterodimers (19), raises the possibility that PDZ proteins could organize the scaffold network in the apical membrane of renal proximal tubular cells (Fig. 3). The existence of PDZ scaffold networks would contribute a "road sign" that is recognized as the final destination of protein sorting and/or the maintenance of epithelial polarity (49). PDZK1 should organize the array of OATs in the apical membrane in the proximal tubular cells and regulate organic anion transport via URAT1, OAT4, MRP2, MRP4, and NPT1 in situ as a functional unit for OA transport in the proximal tubular cells.

MOLECULAR PHYSIOLOGY OF URATE TRANSPORT

Urate is a typical endogenous OA, whose transport in the proximal tubules has been one of major subjects in renal physiology for OA transport. Recently, several potential candidate urate transporters have been identified.

The Proximal Tubule as Regulator of Serum Urate Level

The serum urate level is regulated at 200–500 μM mainly by renal proximal tubular cells. From pharmacological studies using uricosuric and antiuricosuric drugs, a putative renal transport model for urate in humans was proposed. This "four-

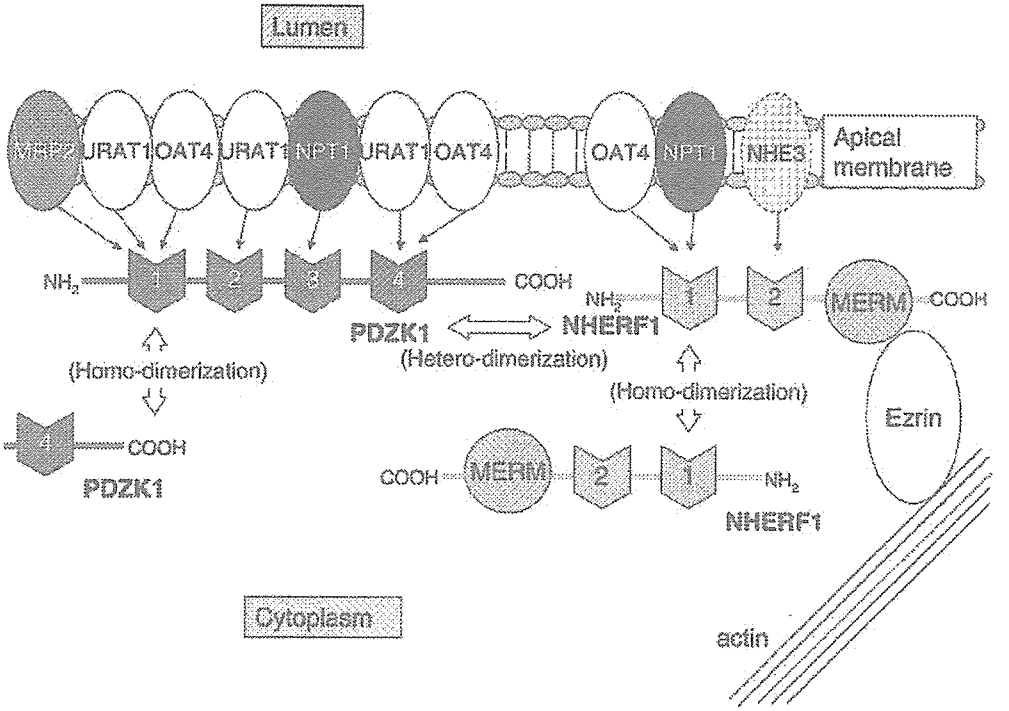

Fig. 3. Schematic presentation of the interaction between PDZ proteins and OATs. Several OATs expressed in the apical membrane of proximal tubular cells are reported to interact with 2 PDZ proteins, namely, Na^+/H^+ exchanger regulatory factor (NHERF) 1 and PDZK1. NHERF1 contains 2 tandem PDZ domains and interacts with NPT1 and OAT4 via PDZ domain 1 (19, 81). NHERF1 also interacts with actin-binding proteins of the merlin-ezrin-radixin-moesin family via a MERM binding lesion at its COOH terminus. PDZK1 contains 4 tandem PDZ domains. The COOH terminus of URAT1 and OAT4 bind with PDZK1. URAT1 has been shown to interact with PDZK1 via PDZ *domains 1*, *2*, and *4*. OAT4 interacts with PDZ *domains 1* and *4* (19, 81). NPT1 and MRP2 also interact with PDZK1. PDZK1 forms a homodimer, and a recent study demonstrated that NHERF1 and PDZK1 interact with each other, raising the possibility that they form a broad scaffolding network that contributes to the stabilization and functional regulation of OATs at the apical membrane of proximal tubular cells.

component model" of urate transport consists of four steps: *1*) glomerular filtration, *2*) reabsorption, *3*) secretion, and *4*) postsecretory reabsorption (61). This complex model had long been an established concept; however, a novel interpretation has recently emerged (54). In the above-mentioned four-component model, secretion was presumed to be mediated by distinct molecules from reabsorption molecules. Pyrazinoate (PZA), which is an active metabolite of pyrazinamide and has an antiuricosuric effect, has been considered to show an antiuricosuric effect mainly by inhibiting the secretory pathway for urate via a voltage-driven urate transporter in the apical membrane. However, recent studies using apical membrane vesicles of human proximal tubules indicated that the effect of PZA on urate transport is mediated by *trans*-stimulation for urate reabsorption via a urate/anion exchanger (54). PZA enters proximal tubular cells via a Na^+-nicotinate cotransporter. Thereafter, PZA stimulates urate uptake via a urate/anion exchanger in the apical membrane of human proximal tubular cells, which reabsorbs urate in exchange for intracellular OAs and inorganic anions, such as lactate, nicotinate, and PZA. According to this model, human proximal tubule secretion, if present, is a minor component of the transepithelial transport of urate (21, 54).

Urate Transporters

Recently, human (h) (14) and mouse (m)URAT1 (24, 28), human (26) and rat OAT1 (57), human OAT3 (4), pig OATv1 (30), human and rat UAT (41), and human MRP4 (74) had been shown to transport urate. Among them, only URAT1 has been clarified by its distinct physiological and pathophysiological roles.

Apical transporters. URAT1. hURAT1 is localized in the apical membrane of proximal tubular cells. hURAT1 mediates the exchange of urate for several OAs and inorganic anions, such as lactate and PZA (14). The functional characteristics of hURAT1 are identical to those of the long-postulated urate/anion exchanger in human renal epithelial cells. The role of hURAT1 in urate handling was verified by genetic analysis in patients with idiopathic renal hypouricemia. Patients with this disorder manifest extremely low levels of serum urate, mostly <2.0 mg/dl. For Japanese patients, 80–90% (27, 37) with hereditary renal hypouricemia have been shown to possess homozygous or compound heterozygous mutations in the hURAT1 gene.

Recently, a mouse ortholog of hURAT1 has been identified and characterized (24, 28). Mouse renal-specific transporter (RST), which was identified as an RST with unknown functions, has a 74% amino acid identity with hURAT1. RST transports urate (K_m: 1,213 μM) and is *cis*-inhibited by probenecid, benzbromarone, and lactate. The substitution of the Cl^- with gluconate in the bath media enhances RST-mediated urate transport, and preinjected PZA or l-lactate *trans*-stimulated RST-dependent urate transport (24). These indicate that RST is

a mouse ortholog of hURAT1. The RST mRNA and protein levels were higher in the male kidneys than female (24). This observation could explain the fact that the serum urate level is higher in males than that in females.

OATv1. Originally, OATv1 was expression-cloned as a voltage-driven PAH transporter from pig kidney (30). OATv1 consists of 467 amino acid residues and exhibits a 60–65% amino acid sequence identity to human, rat, rabbit, and mouse NPT1, which belongs to the *SLC17A* family. OATv1 is localized at the apical membrane of renal proximal tubules. OATv1 also mediates urate transport. The membrane localization and transport properties of urate by OATv1 suggest that OATv1 is a voltage-driven urate transporter, which functions in urate excretion in species of urate secretors, such as pigs and rabbits.

UAT. Another possible urate transporter is UAT (41). UAT was identified by screening a rat kidney cDNA library with a polyclonal antibody to pig liver uricase. UAT was also designated galectin 9, because of its homology to other members of the galectin family, which function in cell-cell interaction and mediation of apoptosis, and as a tumor antigen. UAT/galectin 9 is expressed ubiquitously and localized at the apical side of renal proximal tubular cells (25). Because of its ubiquitous expression and several experimental data, UAT/galectin 9 is speculated to be a housekeeping urate channel that serves in the efflux of urate produced by intracellular purine metabolism (39). The role of UAT/galectin 9 as a urate transporter/channel in the apical membrane of proximal tubules remains to be elucidated.

MRP4. Van Aubel et al. (74) demonstrated that human MRP4, but not MRP2, mediates ATP-dependent urate transport (K_m: 1.5 mM). Urate inhibits methotrexate transport (IC_{50}: 235 μM) by MRP4. Interestingly, MRP4 transports urate simultaneously with cAMP or cGMP, suggesting that MRP4 is a unidirectional efflux pump for urate with multiple allosteric substrate-binding sites. Because of the basolateral expression of MRP4 in hepatocytes, the authors suggested the role of MRP4 as a hepatic exporter for pumping urate into the circulatory system (74).

Basolateral transporters. OAT1 AND OAT3. OAT1 and OAT3, which are localized to the basolateral membrane of proximal tubular cells, were shown to transport urate. Human OAT1 transports urate with a K_m of 943 μM (26). hOAT1-mediated transport of urate is inhibited by uricosuric and antiuricosuric agents, such as benzbromarone, probenecid, salicylate, and PZA. Rat OAT1 also transports urate (57). Bakhiya et al. (4) demonstrated that the hOAT3-mediated efflux of glutarate is significantly *trans*-stimulated by urate (167%), as well as glutarate (282%), α-ketoglutarate (476%), and PAH (179%). This result indicates that hOAT3 functions as a urate/dicarboxylate exchanger. Urate inhibits ES uptake via hOAT3, with an IC_{50} close to the normal serum urate concentration, suggesting that it contributes to urate transport similar to hURAT1. rbOAT3 also mediates the uptake transport of urate (80). The fact that preloaded urate in rbOAT3-expressing cells show a *trans*-stimulatory effect on rbOAT1-mediated transport of urate indicates that rbOAT3 transports urate bidirectionally (80). Taken together, OAT1 and OAT3 appear to serve for urate uptake from plasma into proximal tubular cells; however, the direction of urate transport via OAT1 and OAT3 still remains to be elucidated.

Summary of Urate Transporters with Reference to Species Difference

Figure 2 depicts the putative players in renal urate transport in proximal tubular cells. From the results of previous studies, it was predicted that a urate/anion exchanger exists only in the kidney of a urate reabsorber, whereas the potential-driven pathway is present in both urate secretors and reabsorbers (52). In humans, hURAT1 acts as a urate/anion exchanger mediating urate reabsorption. In the pig, the ortholog of hURAT1 has not been identified, but, if it is present, its role in urate transport might be different from that of hURAT1. OATv1 would function as a voltage-driven urate secretion in pigs. In humans, the putative ortholog of OATv1 is NPT1, and urate efflux via NPT1 is presumably modest. The distinct expression and transport properties of each urate transporter should determine the species difference in the renal handling of urate. The physiological significance of other urate transporters, OAT1, OAT3, UAT, and MRP4, remains to be elucidated.

TRANSEPITHELIAL TRANSPORT OF OAs

At present, the role of each OA transporter in the renal secretory pathway for OA remains to be elucidated. In particular, those in the apical membrane are far from being completely understood. In this final section, we will summarize the present knowledge about OA transporters in the light of the transepithelial transport of OA.

Basolateral Uptake Contribution of OAT1 and OAT3

For the basolateral uptake of type I OA in proximal tubular cells, OAT1 and OAT3 could explain most of the transport. The transport properties of OAT1 are identical to those characterized for the classic basolateral pathway for OA. OAT1 is predominant in PAH uptake, and OAT1 has been presumed to be the major OA transporter in the basolateral membrane of proximal tubular cells. However, accumulated results suggested that OAT3 should be predominant for many important OAs. OAT3 shows a similar wide substrate selectivity to OAT1, and OAT3 mediates the accumulative transport of OA against an electrochemical gradient. Recent studies using OAT3 knockout mice clearly demonstrated the important role of OAT3 in the kidney (65). In the renal slices from OAT3 knockout mice, uptake of ES and taurocholate was essentially reduced to the basal level, indicating that basolateral uptake of taurocholate and ES by renal tubules is mediated mostly by OAT3 in mice. With respect to PAH, uptake was substantially reduced in renal slices from OAT3 knockout mice.

A recent study in humans demonstrates that the elimination constant for cefazolin significantly correlates with phenolsulfonphthalein test and hOAT3 mRNA levels (56). The expression level of OAT3 is higher than that of OAT1 in humans. Zhang et al. (80) demonstrated that the contribution of OAT1 and OAT3 is nearly equal in uptake of ochratoxin A in rabbit renal proximal tubules. They also reported that OAT3 plays a large or larger role than OAT1 and OCTs in cimetidine transport in rabbit proximal tubules. These results indicate that OAT3 plays a predominant role in the transport of several type I OAs rather than OAT1.

Apical Exit

The apical exit pathway for OAs has been much less intensively studied than the basolateral uptake pathway. Marked species differences in apical exit are observed among OAs, as is the case for the urate transport system. Because proximal tubular cells have electrically interior negative conditions compared with the lumen, the cell-to-lumen efflux of OA is energetically downhill. Two transporter-mediated systems have been proposed for this efflux (52). One is an electrically neutral anion/anion exchanger, and the other one is a potential-driven efflux pathway. An anion/anion exchange system is only detected in urate reabsorbers in species such as rats, humans, and mongrel dogs, whereas the potential-driven efflux pathway is present in both urate reabsorbers and urate secretors (52). Another important point is that these systems would be common for the urate transport system in some species, but different in other species (52).

URAT1, OAT4, Oatp1a3v1 (formerly: OAT-K1), and Oatp1a3v2 (formerly: OAT-K2) are candidate anion exchangers in apical membrane molecules. However, there are no human orthologs for Oatp1a3v1 and Oatp1a3v2 for the voltage-driven efflux pathway; OATv1 (NPT1) is a potential candidate. MRP2 and MRP4 were revealed to transport several type I OAs, although physiological information on the primary active transport of type I OA in the apical membrane is very limited.

URAT1 as an apical exit pathway for OA. As discussed in the previous section, URAT1 is a urate transporter in the human kidney. Recent studies suggested another distinct role of URAT1 (28). mURAT1 (the mouse homolog of hURAT1) exhibits a potential-driven saturable uptake of PAH (K_m: 234 mM). An increase in K^+ concentration enhanced the uptake of benzylpenicillin (PC-G), 2,4-dichlorophenoxyacetate, and dehydroepiandrosterone sulfate via mURAT1, suggesting the wide substrate selectivity of mURAT1. In LLC-PK_1 cells coexpressing mURAT1 and rat Oat3, the basal-to-apical transport values of PC-G and urate were 3- and 2.5-fold greater than that in the opposite direction in these double-transfected cells, respectively, suggesting that mURAT1 mediates the cell-to-lumen efflux of various OAs across the apical membrane. hURAT1 actually functions in the reabsopriton of urate from the glomerular filtrate, because its physiological role was proved by mutation analysis in patients with hereditary renal hypouricemia (14). However, the direction of urate transport would also depend on the serum urate concentration, which differs remarkably among species with or without uricase activity. Human uricase activity was lost during the evolutionary process by an inactivating uricase gene, whereas many species including mice retain uricase activity, resulting in extremely low serum urate levels. This fact implies a different physiological role of URAT1 in humans and mice. Species difference is an important problem considering the role of the urate transporter, especially for URAT1 and OATv1. Precise molecular knowledge about these transporters and in vito studies addressing this issue will clarify the role of individual transporters related to OA efflux.

OATv1. OATv1 (30) and the putative human ortholog NPT1 (69) transport PAH. Transport of PAH via OATv1 is dependent on membrane potential, and the affinity was low (K_m = 4.4 mM). OATv1 also transports ES and estradiol glucronide, and OATv1-mediated uptake of PAH is inhibited by various anionic compounds including probenecid, salylate, diclofenac, furosemide, and bumetanide. Efflux of preloaded PAH from the cells expressing OATv1 was clearly demonstrated. Human NPT1 also mediates low-affinity transport of PAH (K_m: 2.66 mM) (69). Human NPT1-mediated transport of PAH is inhibited by PC-G, salicylate, indomethacin, and probenecid. Rabbit NPT1 also transports PC-G (K_m: 0.22 mM), and probenecid and phenol red in addition to PCG induce outward currents in oocytes expressing rabbit NPT1 (9). These results indicate that OATv1 and NPT1 function as a voltage-driven OA transporter for type I OAs in the apical membrane of proximal tubular cells.

MRP2 and MRP4. As has been described, MRP2 and MRP4 mediate the ATP-dependent transport of type II OAs (73). These two primary active transporters should play a role in extrusion of several large and hydrophobic OAs from the proximal tubular cells, such as bile acids. With respect to the role in efflux of type I OAs, their contribution remains to be elucidated.

CONCLUDING REMARKS

In this review, we describe recent knowledge about the physiological roles of OA transporters, especially OAT family members. With respect to the roles of OATs, their pharmacological significance has been emphasized, because of wide substrate selectivity including clinically important acidic drugs. Molecular findings on OATs are very useful in the study of pharmacokinetics and drug-drug interactions. However, recent studies unveiled gender differences and the reabsorptive roles of endogenous compounds, such as urate for OAT isoforms. This implies that the fundamental roles of OATs are not restricted to pharmacological and toxicological roles. This is further supported by the fact that hURAT1, a member of the OAT family, is responsible for a human disease, hereditary renal hypouricemia, and that OAT expression is affected by various pathophysiological states. The defect in OCTN2, other member of the *SLC22A* gene family, causes systemic carnitine deficiency. OCTN1, another member of the *SLC22A* family, has also been revealed to be associated with inflammatory diseases, such as Crohn's disease and rheumatoid arthritis. Involvement of OATs in various pathophysiological conditions are another important issue. This type of information is important in the following context. First, it reveals the relationship of OATs in the development of pathophysiogocal states such as renal impairment. Second, it might give us some clues as to the physiological roles of OATs. Analysis of single nucleotide polymorphisms in OATs also would give some information.

The history of OA transport systems in the kidneys is very long. However, the physiological roles of each organic anion transporter should be unveiled in further studies.

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Renal expression of organic anion transporter OAT2 in rats and mice is regulated by sex hormones

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Renal expression of organic anion transporter OAT2 in rats and mice is regulated by sex hormones

Marija Ljubojević,[1] Daniela Balen,[1] Davorka Breljak,[1] Marija Kušan,[2] Naohiko Anzai,[3] Andrew Bahn,[4] Gerhard Burckhardt,[4] and Ivan Sabolić[1]

[1]Molecular Toxicology, Institute for Medical Research and Occupational Health, Zagreb, Croatia; [2]Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia; [3]Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan; and [4]Vegetative Physiology and Pathophysiology, University of Göttingen, Göttingen, Germany

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Ljubojević M, Balen D, Breljak D, Kušan M, Anzai N, Bahn A, Burckhardt G, Sabolić I. Renal expression of organic anion transporter OAT2 in rats and mice is regulated by sex hormones. *Am J Physiol Renal Physiol* 292: F361–F372, 2007. First published August 1, 2006; doi:10.1152/ajprenal.00207.2006.—The renal reabsorption and/or excretion of various organic anions is mediated by specific organic anion transporters (OATs). OAT2 (Slc22a7) has been identified in rat kidney, where its mRNA expression exhibits gender differences [females (F) > males (M)]. The exact localization of OAT2 protein in the mammalian kidney has not been reported. Here we studied the expression of OAT2 mRNA by RT-PCR and its protein by Western blotting (WB) and immunocytochemistry (IC) in kidneys of adult intact and gonadectomized M and F, sex hormone-treated castrated M, and prepubertal M and F rats, and the protein in adult M and F mice. In adult rats, the expression of OAT2 mRNA was predominant in the outer stripe (OS) tissue, exhibiting *1*) gender dependency (F > M), *2*) upregulation by castration and downregulation by ovariectomy, and *3*) strong downregulation by testosterone and weak upregulation by estradiol and progesterone treatment. A polyclonal antibody against rat OAT2 on WB of isolated renal membranes labeled a ~66-kDa protein band that was stronger in F. By IC, the antibody exclusively stained brush border (BB) of the proximal tubule S3 segment (S3) in the OS and medullary rays (F > M). In variously treated rats, the pattern of 66-kDa band density in the OS membranes and the staining intensity of BB in S3 matched the mRNA expression. The expression of OAT2 protein in prepubertal rats was low and gender independent. In mice, the expression pattern largely resembled that in rats. Therefore, OAT2 in rat (and mouse) kidney is localized to the BB of S3, exhibiting gender differences (F > M) that appear in puberty and are caused by strong androgen inhibition and weak estrogen and progesterone stimulation.

androgens; estrogens; gender differences; kidney; membrane transporters; progesterone; organic anion; transporter-2

PROCESSES OF reabsorption, distribution, and elimination of endogenous and xenobiotic organic anions (OA) in the mammalian liver and kidney are mediated by the multispecific OA transporters (OATs; subfamily of Slc22 drug transporters) that reside in the apical or basolateral membrane of epithelial cells (reviewed in Refs. 9, 18, 36, 46, 48, 52, 53, 55–57). In humans and experimental animals, the rate of renal excretion of many OA exhibits gender differences, which, at least in rodents, can be correlated with the sex-dependent expression of relevant OATs at the level of mRNAs and/or proteins (6–8, 10, 19, 21, 27, 29, 32, 37, 41, 46, 47, 49, 54, 55).

OAT2 (Slc22a7) was originally cloned from the rat liver cDNA as novel liver-specific transporter (NLT) (50), later renamed OAT2 (48). The rat ortholog of OAT2 (rOAT2) consists of 535 amino acid residues, with intracellular COOH and NH_2 terminals and two possible N-glycosylation sites (50), and exhibits 87 and 79% identity to mouse (mOAT2) (27) and human OAT2 (hOAT2) (50) as well as 42 and 39% homology to rOAT1 (48) and rOAT3 (30), respectively. When expressed in *Xenopus laevis* oocytes or cultured cells, rOAT2 mediated the Na^+-independent transport of salicylate, acetylsalicylate, prostaglandin E_2, α-ketoglutarate, methotrexate, and (weakly) *p*-aminohippuric acid (PAH), whereas hOAT2 and mOAT2 did not transport salicylate, indicating subtle species differences in the substrate specificity (27, 48, 51). The human and rat OAT2 may also transport some prostaglandins, cephalosporin antibiotics, and some other chemotherapeutics (9, 13, 22, 36). The driving force(s) for OAT2-mediated transports has not been clearly resolved; although recently claimed to be an OA/dicarboxylate (fumarate, succinate) exchanger (26), its definition as an anion exchanger or facilitator is still disputable.

In rats, mice, and humans, the OAT2-specific mRNA is principally expressed in liver and kidney, showing somehow contradictory data regarding its level in different species and studies. In rats, a low level of rOAT2 expression was detected in fetal time and before puberty (<40 days of age) in both organs (6, 50), whereas in adult animals, the expression in male (M) and female (F) kidney was found to be either lower (50) or higher (6, 27) than in liver. The rOAT2 expression in kidney exhibited strong gender differences (F > M), possibly due to growth hormone-mediated inhibitory action of androgens (6, 7, 24, 29). In mice, mOAT2 expression was also low in the fetal and prepubertal period (8, 39), whereas in sexually mature animals, the expression in kidney was found to be either much lower (39) or much higher (8, 27) than in liver; gender differences were observed in liver (F > M) by Kobayashi et al. (27) but not by Buist and Klaassen (8) and not in kidney (8, 27). In humans, hOAT2 appears in two alternatively spliced forms (hOAT2A and hOAT2B) showing different COOH-terminal sequences that are expressed more in liver than kidney (51).

While the rOAT2 protein [relative molecular mass (M_r) ~62 kDa] in the rat liver cells was immunolocalized to the sinusoidal membrane (50), the exact localization of this transporter in the mammalian kidney is still controversial. In the study by

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