Molecular physiology of renal organic anion transporters

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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 proteins; 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 glucuronide 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.
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 \textit{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, PGE2, 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 to the apical 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 to the apical membrane of proximal tubules (2). In the placenta, OAT4 is expressed on the...
Enzymes. *Expression level of OAT2 shows gender differences in the liver (female vs. male), whereas no significant difference is 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.

Table 1. Members of the OAT family

<table>
<thead>
<tr>
<th>Gene Product (Gene Symbol)</th>
<th>Identified Species</th>
<th>Gender Difference</th>
<th>Tissue Distribution</th>
<th>Transport Mechanism</th>
<th>Membrane Localization in PT</th>
<th>Representative Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAT1 (SLC22A6)</td>
<td>Caenorhabditis elegans, flounder, human, mouse, pig, rabbit, rat</td>
<td>Yes (rat) Male &gt; female</td>
<td>Brain, kidneys, placenta, eyes, smooth muscle</td>
<td>OA/DC exchanger</td>
<td>Basolateral</td>
<td>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</td>
</tr>
<tr>
<td>OAT2 (SLC22A7)</td>
<td>Human, mouse, rat</td>
<td>Yes (mouse)*</td>
<td>Kidneys, liver</td>
<td>Unknown</td>
<td>†</td>
<td>PAH, DC, PGE2, salicylate, MTX, acetylsalicylate</td>
</tr>
<tr>
<td>OAT3 (SLC22A8)</td>
<td>Human, rabbit, mouse, pig, rat</td>
<td>Yes (rat) Male &gt; female</td>
<td>Bone, brain, eyes, kidneys, liver, adrenal glands</td>
<td>OA/DC exchanger</td>
<td>Basolateral</td>
<td>ES, DHEA-S, PAH, DC, urate, cyclic nucleotides, cortisol, cimetidine, salicylate, uremic toxins, MTX, β-lactam antibiotics, ochratoxin A, PAH, DHEA-S, PAG, PGE2, PGF3α</td>
</tr>
<tr>
<td>OAT4 (SLC22A11)</td>
<td>Human</td>
<td>Unknown</td>
<td>Kidneys, placenta</td>
<td>OA/DC exchanger</td>
<td>Apical</td>
<td>ES, DHEA-S, PAH, ochratoxin A, PGE2, PGF3α</td>
</tr>
<tr>
<td>URAT1 (SLC22A12)</td>
<td>Human, mouse</td>
<td>Yes (mouse)*</td>
<td>Brain, kidneys</td>
<td>Urate/anion exchanger</td>
<td>Apical</td>
<td>Urate</td>
</tr>
<tr>
<td>OAT5 (SLC22A19)</td>
<td>Mouse, rat</td>
<td>Male &gt; female</td>
<td>Kidneys</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Ochratoxin A</td>
</tr>
</tbody>
</table>

OA, organic anion; OAT, OA transporter; PAH, p-aminophenol; DC, dicarboxylates; ES, estrone sulfate; DHEA-S, dehydroepiandrosterone sulfone; 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 is 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.

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

<table>
<thead>
<tr>
<th>Gene Product (Gene Symbol)</th>
<th>Old Name</th>
<th>Identified Species</th>
<th>Tissue Distribution</th>
<th>Int intrarenal Membrane Localization</th>
<th>Representative Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatp1a1 (Scl01a)</td>
<td>Oatp1</td>
<td>Mouse, rat</td>
<td>Brain, colon, kidneys, liver, lungs, small intestine</td>
<td>PT (S3): apical</td>
<td>BSP, taurocholate, E17βG, LTC4, DNP-SG, T3, T4, aldosterone, cortisol, ouabain, ochratoxin A, temocaprilat, enalapril</td>
</tr>
<tr>
<td>OATP1A2 (SLCO1A2)</td>
<td>OATP-A</td>
<td>Human</td>
<td>Brain, kidneys, liver</td>
<td>CCD: basolateral</td>
<td>BSP, chololate, taurocholate, DHEA-S, E17βG, PGE3, T3, T4, chlorambucil, fexofenadine, ouabain, BQ123, CRC220, ochratoxin A</td>
</tr>
<tr>
<td>Oatp1a3 v1 (Scl0a3)</td>
<td>Oat-K1</td>
<td>Rat</td>
<td>Kidneys</td>
<td>PT (S3): apical</td>
<td>Taurocholate, E17βG, ES, DHES, folate, T3, T4, MTX</td>
</tr>
<tr>
<td>Oatp1a3 v2 (Scl0a3)</td>
<td>Oat-K2</td>
<td>Rat</td>
<td>Kidneys</td>
<td>PT + CCD: apical</td>
<td>Taurocholate, E17βG, ES, DHES, folate, T3, T4, MTX</td>
</tr>
<tr>
<td>Oatp1a5 (Scl0a7)</td>
<td>Oatp3</td>
<td>Mouse, rat</td>
<td>Kidneys, lungs, retina</td>
<td>Unknown</td>
<td>Taurocholate, T3, T4</td>
</tr>
<tr>
<td>OATP2A1 (SLCO2A1)</td>
<td>PGT</td>
<td>Human, rat</td>
<td>Ubiquitous</td>
<td>Unknown</td>
<td>PGs</td>
</tr>
<tr>
<td>OATP2B1 (SLCO2B1)</td>
<td>OATP-B</td>
<td>Human</td>
<td>Brain, heart, intestine, kidneys, liver, placenta</td>
<td>Unknown</td>
<td>Taurocholate, E17βG, ES, PGE2, T3, T4, PC-G</td>
</tr>
<tr>
<td>OATP3A1 (SLCO3A1)</td>
<td>OATP-D</td>
<td>Human, mouse</td>
<td>Ubiquitous</td>
<td>Unknown</td>
<td>ES, PGE2, PC-G</td>
</tr>
<tr>
<td>OATP4A1 (SLCO4A1)</td>
<td>OATP-E</td>
<td>Human, mouse, rat</td>
<td>Ubiquitous</td>
<td>Unknown</td>
<td>Taurocholate, E17βG, ES, PGE2, T3, T4, PC-G</td>
</tr>
<tr>
<td>OATP4C1 (SLCO4C1)</td>
<td>OATP-H</td>
<td>Human, rat</td>
<td>Kidneys</td>
<td>PT: basolateral</td>
<td>T3, digoxin, ouabaine</td>
</tr>
<tr>
<td>Oatp1a6 (Scl0l6)</td>
<td>Oatp5</td>
<td>Mouse, rat</td>
<td>Kidneys</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

OATP, OA-transporting peptide; BSP, bromosulfophthalein; E17βG, estradiol 17β-o-glucuronide; LTC4, leukotrien C4; DNP-SG, S-(dinitrophenyl)-glutathione; PC-G, benzylpenicillin; CCD, cortical collecting duct.
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

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

<table>
<thead>
<tr>
<th>Gene Product (Gene Symbol)</th>
<th>Identified Species</th>
<th>Tissue Distribution</th>
<th>Intrarenal and Membrane Localization</th>
<th>Representative Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 (ABCB1)</td>
<td>Human, mouse, rat</td>
<td>Brain, intestine, kidneys</td>
<td>PT: apical</td>
<td>ES, DHEA-S, E\textsubscript{2} 17βG, rhodamine123, anti-HIV agents, anticancer agents, verapamil, fluo 3, calcine, cardiac glycosides</td>
</tr>
<tr>
<td>MRP1 (ABCC1)</td>
<td>Human, mouse</td>
<td>Ubiquitous</td>
<td>MTAL–CCD: basolateral</td>
<td>LTC\textsubscript{a}, DNP-SG, E\textsubscript{2} 17βG, GSSG, AFB\textsubscript{1}-SG, PGA\textsubscript{1}-SG, PGA\textsubscript{2}-SG, GSH, PAH, MTX, etoposide-G, fluo 3, calcine</td>
</tr>
<tr>
<td>MRP2 (ABCC2)</td>
<td>Human, mouse, rat</td>
<td>Brain, intestine, kidneys, liver</td>
<td>PT: apical</td>
<td>LTC\textsubscript{a}, DNP-SG, E\textsubscript{2} 17βG, folate, glycholate, urate, cAMP, CGMP, PMEA, AZTMP, MTX, PAH, etoposide-G, fluo 3, calcine</td>
</tr>
<tr>
<td>MRP3 (ABCC3)</td>
<td>Human, rat</td>
<td>Adrenals, intestine, kidneys, liver, pancreas</td>
<td>CCD: basolateral</td>
<td>LTC\textsubscript{a}, DNP-SG, E\textsubscript{2} 17βG, folate, glycholate, MTX</td>
</tr>
<tr>
<td>MRP4 (ABCC4)</td>
<td>Human, rat</td>
<td>Ubiquitous</td>
<td>PT: apical</td>
<td>E\textsubscript{2} 17βG, urate, cAMP, CGMP, PMEA, AZTMP, MTX, adefovir.</td>
</tr>
<tr>
<td>MRP5 (ABCC5)</td>
<td>Human</td>
<td>Ubiquitous</td>
<td>Unknown: basolateral</td>
<td>GSH, cAMP, cGMP, 6-MP, adefovir, DNP-SG, CMFDA, BCCF, FDA</td>
</tr>
<tr>
<td>MRP6 (ABCC6)</td>
<td>Human</td>
<td>Kidneys, liver</td>
<td>PT: basolateral</td>
<td>LTC\textsubscript{a}, NEM-SG, BQ123</td>
</tr>
</tbody>
</table>

MRP, multidrug resistance-associated protein; AFB\textsubscript{1}-SG, S-(aflatoxin B\textsubscript{1})-glutathione; AZTMP, azidothymidine monophosphate; CMFDA, 5-chloromethylfluorescein; 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.

Invited Review
Molecular Physiology of the Organic Anion Transporter

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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 glucuronides 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 phosphorylation 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 PKA attenuated the cellular uptake of estrone sulfate (ES) in OAT3-
expressing cells in a dose- and time-dependent manner. PMA treatment decreased $V_{\text{max}}$ 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 phosphorylation of mouse OAT1 (for a more extensive overview on this matter, see Ref. 68).

Pathophysiological States

Recent studies indicated that the expressions of OATs are affected in pathophysiologic 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 dysfunction, and this also accelerates underlying renal diseases. OA; their accumulation in the kidney is a result of renal proteins accumulate in uremic plasma. Many uremic toxins are.

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 chromosome 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).

Genomic Organization and Possible Transcriptional Regulation

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 Φ 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 (NHERF1) have 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-
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+/H+–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, benz bromarone, 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 galecin 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 $\mu$M) 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 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.

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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 $\mu$M (26). hOAT1-mediated transport of urate is inhibited by uricosuric and antiuricosuric agents, such as benz bromarone, probenecid, salicylate, and PZA. Rat OAT1 also transports urate (57). Bakhinya et al. (4) demonstrated that the hOAT3-mediated efflux of glutarate is significantly trans-stimulated by urate (167%), as well as glutarate (282%), $\alpha$-ketoglutarate (476%), and PAH (179%). This result indicates that hOAT3 functions as a urate/dicarbonyl oxidase exchangeur. 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 phenolsulfophthalein 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 humanorthologs 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-PK1 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 reabsorption of urate from the glomerular filtrate, because its physiological role was proved 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 efflux 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 pathophysiologic 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 pathophysiologic 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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