invited review

Molecular pharmacology of renal organic anion transporters

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Van Aubel, Rémon A. M. H., Rosalinde Masereeuw, and Frans G. M. Russel. Molecular pharmacology of renal organic anion transporters. Am J Physiol Renal Physiol 279: F216–F232, 2000.—Renal organic anion transport systems play an important role in the elimination of drugs, toxic compounds, and their metabolites, many of which are potentially harmful to the body. The renal proximal tubule is the primary site of carrier-mediated transport from blood to urine of a wide variety of anionic substrates. Recent studies have shown that organic anion secretion in renal proximal tubule is mediated by distinct sodium-dependent and sodium-independent transport systems. Knowledge of the molecular identity of these transporters and their substrate specificity has increased considerably in the past few years by cloning of various carrier proteins. However, a number of fundamental questions still have to be answered to elucidate the participation of the cloned transporters in the overall tubular secretion of anionic xenobiotics. This review summarizes the latest knowledge on molecular and pharmacological properties of renal organic anion transporters and homologs, with special reference to their nephron and plasma membrane localization, transport characteristics, and substrate and inhibitor specificity. A number of the recently cloned transporters, such as the p-aminohippurate/dicarboxylate exchanger OAT1, the anion/sulfate exchanger SAT1, the peptide transporters PEPT1 and PEPT2, and the nucleoside transporters CNT1 and CNT2, are key proteins in organic anion handling that possess the same characteristics as has been predicted from previous physiological studies. The role of other cloned transporters, such as MRP1, MRP2, OATP1, OAT-K1, and OAT-K2, is still poorly characterized, whereas the only information that is available on the homologs OAT2, OAT3, OATP3, and MRP3–6 is that they are expressed in the kidney, but their localization, not to mention their function, remains to be elucidated.

multidrug resistance protein; peptide transporter; drug excretion; proximal tubule; kidney

The human body is continuously exposed to a great variety of xenobiotics, via food, drugs, occupation, and environment. Excretory organs such as kidney, liver, and intestine defend the body against the potentially harmful effects of these compounds by biotransformation into less active metabolites and excretory transport processes. Most drugs and environmental toxicants are eventually excreted into the urine, either in the unchanged form or as biotransformation products. The mechanisms that contribute to their renal excretion are closely related to the physiological events occurring in the nephrons, i.e., filtration, secretion, and reabsorption. Carrier-mediated transport of xenobiotics and their metabolites is confined to the proximal tubule, and separate carrier systems exist for the active secretion of organic anions and cations. Both systems are characterized by a high clearance capacity and tremendous diversity of substances accepted, prob-
ably resulting from multiple transporters with overlapping substrate specificities.

This review will focus on the molecular aspects of renal organic anion transporters. These systems play a critical role in the elimination of a large number of drugs (e.g., antibiotics, chemotherapeutics, diuretics, nonsteroidal anti-inflammatory drugs, radioccontrast agents, cytostatics); drug metabolites (especially conjugation products with glutathione, glucuronide, glycin, sulfate, acetate); and toxicants and their metabolites (e.g., mycotoxins, herbicides, plasticizers, glutathione S-conjugates of polyhaloalkanes, polyhaloalkenes, hydroquinones, aminophenols), many of which are specifically harmful to the kidney. For a review of the molecular pharmacology of renal organic cation transporters, the reader is referred to a recent comprehensive paper by Koepsell et al. (79). The purpose of this paper is to give a concise review of the latest molecular information on organic anion transporters that contribute to the renal handling of xenobiotics and their metabolites. These transporters are depicted in Fig. 1 and listed in Tables 1 and 2. The systems involved in organic anion secretion can be functionally subdivided in the well-characterized sodium-dependent \( p \)-aminohippurate (PAH) system and a recently discovered sodium-independent system (107, 108, 146). Both systems mediate two membrane translocation steps arranged in series: uptake from blood across the basolateral membrane of renal epithelial cells followed by efflux into urine across the apical membrane. While transported through the cytoplasm, substrates for both systems also accumulate in intracellular compartments. Furthermore, at the apical membrane several transport systems have been identified that are involved in reabsorption of anionic xenobiotics. Recent discoveries on the molecular identity of some of the renal organic anion transporters [OAT1; Na\(^+\)-dicarboxylate cotransporter (SDCT2); anion/sulfate exchanger (SAT1); peptide transporters (PEPT1 and PEPT2); nucleoside transporters (CNT1 and CNT2)] have confirmed the characteristics that have been predicted creatively by earlier functional studies performed in isolated membrane vesicles and proximal tubules. The same studies have indicated the existence of further anion transporters that have not yet been identified at the molecular level. For other recently cloned organic anion transporters, information on their function [multidrug resistance protein (MRP) 1, MRP2, OATP1, OAT-K1, OAT-K2] and renal localization (OAT2, OAT3, MRP3–6) remains to be elucidated. Emphasis in this review is being placed on molecular characteristics, nephron and plasma membrane localization, transport properties, and substrate and inhibitor specificity of cloned renal organic anion transporters and homologs.

![Fig. 1. Schematic model of organic anion transporters in renal proximal tubule. Uptake of organic anions (OA\(^-\)) across the basolateral membrane is mediated by the classic sodium-dependent organic anion transport system, which includes \( \alpha \)-ketoglutarate \( (\alpha-KG^-) \)/OA\(^-\) exchange via the organic anion transporter (OAT1) and sodium-ketoglutarate cotransport via the Na\(^+\)/dicarboxylate cotransporter (SDCT2). A second sodium-independent uptake system for bulky OA\(^-\) has recently been identified, but its molecular identity is unknown. Intracellular accumulation occurs for substrates of both transport systems in mitochondria and vesicles of unknown origin. The apical (brush-border) membrane contains various transport systems for efflux of OA\(^-\) into the lumen or reabsorption from lumen into the cell. The multidrug resistance transporter, MRP2, mediates primary active luminal secretion. The organic anion transporting polypeptide, OATP1, and the kidney-specific OAT-K1 and OAT-K2 might mediate facilitated OA\(^-\) efflux but could also be involved in reabsorption via an exchange mechanism. PEPT1 and PEPT2 mediate luminal uptake of peptide drugs, whereas CNT1 and CNT2 are involved in reabsorption of nucleosides.](http://ajprenal.physiology.org/)
## Table 1. Molecular characteristics of organic anion transporters in the kidney

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<th>Name</th>
<th>Alternative Name</th>
<th>Species</th>
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<th>Chromosome Localization</th>
<th>M, kDa</th>
<th>mRNA, kb</th>
<th>TM</th>
<th>Tissue Distribution</th>
<th>Nephron Distribution</th>
<th>Membrane Localization</th>
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(ROAT, renal organic anion transporter; hPAHT, human p-aminohippurate (PAH) transporter; NKT, novel kidney transporter; NLT, novel liver transporter; Sat1, sulfate anion transporter; MRP, multidrug resistance protein; cMOAT, canalicular multispecific organic anion transporter; cMRP, canalicular MRP; MLP, multidrug resistance protein-like protein; Oatp, organic anion transport poly peptide; Oat-k1/Oat-k2, kidney-specific organic anion transporter 1 and 2; CNT, concentrative nucleotide transporter; PEPT, peptide transporter; PT, transmembrane-spanning domain; BLM, basolateral membrane; BBM, brush-border membrane; PT, proximal tubule; ND, not determined; k, kidney; li, liver; lu, lung; br, brain; si, small intestine; c, colon; t, testis; r, retina; pl, placenta; p, pancreas; n, nerve; du, duodenum; m, muscle; b, bladder; gb, gall bladder; CCD, cortical collecting duct; LH, limb of Henle’s loop; M, molecular weight calculated from the deduced amino acid sequence. *Based on localization in liver (basolateral membranes. †Tissue distribution reported by Kool et al. (82). ‡Tissue distribution reported by Lee et al. (89).
Table 2. Functional characteristics of organic anion transporters in the kidney

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<tr>
<th>Name</th>
<th>Species</th>
<th>Transport Mechanism</th>
<th>Substrates, ( K_m )</th>
<th>Inhibitors, ( K_i )</th>
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<td>Naproxen (2 ( \mu M )); ibuprofen (3.5 ( \mu M )); salicylurate (11 ( \mu M )); piroxicam (52 ( \mu M )); salicylate (341 ( \mu M )); acetylsalicylate (428 ( \mu M )); phenacetin (488 ( \mu M )); paracetamol (2 ( mM )); furosemide; indomethacin (10 ( \mu M )); probenecid; urate; ( \alpha )-KG; glutarate; MTX; PGE(_2); cAMP; cGMP</td>
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<td>LTC(_4) (0.1 ( \mu M )); E(_2)17βG (1.5 ( \mu M )); DNP-SG (3.6 ( \mu M )); bilirubin-glucuronide; GSSG (93 ( \mu M )); AF(_2)-SG (0.2 ( \mu M )); PGA(_1)-SG; PGA(_2)-SG (1 ( \mu M )); etoposide-glucuronide; S-(ethacrynic acid)-glutathione</td>
<td>MK571 (0.6 ( \mu M )); CsA (5 ( \mu M )); PSC933 (27 ( \mu M )); S-(decyl)-glutathione (0.7 ( \mu M )); GS-DOX (60/120 ( nM )); GS-DAU (70/200 ( nM )); probenecid</td>
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<td>Oatp1</td>
<td>Rat</td>
<td>TC (27 μM); BQ123 (0.27 μM); aldosterone (15 nM); cortisol (13 nM); ouabain (17 mM); octratoxin A (17 μM); thyroxine; triiodo-L-thyronine; enalapril (214 μM); temocaprilat (47 μM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oatp3</td>
<td>Rat</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oat-k1</td>
<td>Rat</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Inhibitors/Activators:**
- **Mrp2:** MK571; CsA; GSH (20 mM)
- **Mrp3:** PAH; lucifer yellow; fluorescein-methotrexate; phenolphthalein-glucuronide; CsA, probenecid
- **Mrp6:** E3040-glucuronide; α-naphthyl-β-D-glucuronide; MTX
- **Oatp1:** Dipyridamole; Sulfinpyrazone
- **Oatp3:** Thyroxine; triiodothyronine
- **Oat-k1:** MTX; BSP; probenecid; PAH; furosemide; valproate; DIDS; TCA; ibuprofen; flufenamate; phenylbutazone; indomethacin (1 mM); ketoprofen (2 mM)
Table 2.—Continued

<table>
<thead>
<tr>
<th>Name</th>
<th>Species</th>
<th>Transport Mechanism</th>
<th>Unconjugated Substrates, $K_m$ (μM)</th>
<th>Conjugated Substrates, $K_m$ (μM)</th>
<th>Inhibitors, $K_i$ (μM)</th>
<th>Reference No(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat-k2</td>
<td>Rat</td>
<td>ND</td>
<td>TC (10 μM); MTX; PGE$_{2\alpha}$; folate</td>
<td></td>
<td>MTX; BSP; probencid; PAH; furosemide; valproate; DIDS; TCA; levofoxacin; indomethacin; testosteron; dexamethasone; 17β-estradiol; ouabain</td>
<td>113</td>
</tr>
<tr>
<td>CNT1</td>
<td>Human</td>
<td>Nucleoside-Na$^+$ symport</td>
<td>Adenosine (15/26 μM); thymidine (13 μM); uridine (26/45); AZT (0.5 μM); dideoxythidine (0.5 μM)</td>
<td>Formycin B, guanosine, adenosine, uridine, inosine, thymidine</td>
<td>Formycin B, guanosine, uridine, inosine, thymidine</td>
<td>35, 59, 152, 207, 208</td>
</tr>
<tr>
<td>Cnt1</td>
<td>Rat</td>
<td>Nucleoside-Na$^+$ symport</td>
<td>Adenosine (8 μM); uridine (40/80 μM); inosine (5 μM)</td>
<td></td>
<td>Formycin B, guanosine, adenosine, uridine, inosine</td>
<td>153, 198</td>
</tr>
<tr>
<td>CNT2</td>
<td>Human</td>
<td>Nucleoside-Na$^+$ symport</td>
<td>Adenosine (6 μM); inosine (20 μM); uridine (20 μM); cladribine, thymidine (13 μM)</td>
<td></td>
<td>Formycin B, guanosine, adenosine, uridine, inosine, thymidine</td>
<td>17, 161</td>
</tr>
<tr>
<td>PEPT1</td>
<td>Human</td>
<td>Peptide-H$^+$ symport</td>
<td>Glycylsarcosine (1.1 mM); ALA (0.28 mM); cefitubutin (0.3 mM); cephalexin (0.5 mM); cefadroxil (1 mM); l-Val-ACV (5 μM); l-Val-AZT; L-dopa-l-Phe; formyl-Met-Leu-Phe</td>
<td></td>
<td>Ampicillin (50 mM); amoxicillin (13 mM); cefalexin (0.17 mM); cephalexin (5 μM); cefadroxil (2 μM); cephradine (9 μM); cefdinir (12 μM); cefitubutin (0.6 μM); cefixime (7 μM); bestatin (0.5 μM); enalapril (4.3 μM); captopril (9 μM); L-Val-ACV (0.74 mM)</td>
<td>4, 30, 42–44, 54, 119, 179, 181, 182, 200</td>
</tr>
<tr>
<td>PepT1</td>
<td>Rat</td>
<td>Peptide-H$^+$ symport</td>
<td>Glycylsarcosine (0.11 mM); ALA (0.23 mM); cephalexin; bestatin; L-Val-ACV</td>
<td></td>
<td>Ampicillin (0.67 mM); amoxicillin (0.18 mM); cefalexin (27 μM); cephalexin (50 μM); cefadroxil (3 μM); cephradine (47 μM); cefdinir (20 μM); cefitubutin (1 μM); cefixime (12 μM); bestatin (20 μM); L-Val-ACV (0.39 μM)</td>
<td>30, 42–44, 182</td>
</tr>
</tbody>
</table>

$K_m$ and $K_i$, Michaelis-Menten and inhibition constant, respectively; OA, organic anion; α-KG, α-ketoglutarate; MTX, methotrexate; PGE$_{2\alpha}$, prostaglandin E$_{2\alpha}$; BSP, bromosulfophthalein; LTC$_4$, leukotriene C$_4$; LTD$_4$, leukotriene D$_4$; E$_{2\alpha}$17βG, estradiol-17β-glucuronide; DNP-SG, dinitorphenyl-glutathione; AFB$_3$-SG, S-(aflatoxin B$_1$)-glutathione; E3040-glucuronide, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl)benzothiazole glucuronide; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; AZTMP, azidothymidine monophosphate; CMFDA, 5-chloromethylfluorescein; FDA, fluorescein-diacetate; BCECF, 2', 7'-bis-(2-carboxyethyl)-5 (and-6)-carboxyfluorescein areoxymethyl ester; TC, taurocholate; AZT, azidothymidine; ALA, delta-aminolevulinic acid; l-Val-ACV, valacyclovir; PGA$_2$-SG, S-(prostaglandin A$_2$)-glutathione; GS-DOX, S-(doxorubicin)-glutathione; GS-DAU, S-(daunorubicin)-glutathione; BQ-123: cyclo-Trp-Asp-Pro-Val-Leu; BQ-485: perhydroazepino-N-carbonyl-(Leu-Trp-Trp); BQ-518: cyclo-(Trp-Asp-Pro-Thg-Leu). Substrates mentioned for some transporters are indicative but not complete.
BASOLATERAL TRANSPORT SYSTEMS

Transport studies using isolated membrane vesicles and intact proximal tubules have identified and characterized the classic transport system for uptake of small organic anions across the basolateral membrane by using PAH or fluorescein as a model substrate (146). These studies have established that uptake of PAH is a tertiary active process by indirect coupling to the Na\(^+\) gradient. This gradient, which is maintained by the Na\(^+\)-K\(^+\) ATPase, drives Na\(^+\)-dicarboxylate cotransport into the cell and enables uptake of PAH in exchange for a dicarboxylate ion. The PAH/dicarboxylate exchanger (Oat1) has been cloned from various species. On the basis of similarities with transport characteristics found in membrane vesicles, SDCT2 has been proposed as the basolateral Na\(^+\)-dicarboxylate cotransporter (18). \(\alpha\)-Ketoglutarate is by far the most abundant potential dicarboxylate counterion within the proximal tubular cell, and it has been shown that PAH or fluorescein uptake in renal proximal tubules increases with increasing internal \(\alpha\)-ketoglutarate concentration (16, 145, 199). The activity of the Na\(^+\)/dicarboxylate exchanger accounts for \(\sim 60\%\) of organic anion uptake (199), whereas the remainder can be explained by intracellularly stored \(\alpha\)-ketoglutarate (145). Mitochondrial metabolism seems responsible for the generation of this dicarboxylate, and from liver studies it is known that the mitochondrial \(\alpha\)-ketoglutarate concentration exceeds that in cytoplasm three- to sixfold (169, 172). These findings imply that alterations in cellular metabolism may have a direct effect on the secretory function of kidney proximal tubules. Apart from the classical PAH transporter, an additional uptake system has been characterized by using the bulky organic anion fluorescein-methotrexate as a substrate (108). Uptake of fluorescein-methotrexate is independent of Na\(^+\) and is not inhibited by PAH or the dicarboxylate ion glutarate. The molecular identity of this transporter, however, has not yet been established. Finally, a sulfate/anion exchanger has been characterized in basolateral membrane vesicles and cloned from rats (52, 72, 85, 147). This transporter exchanges sulfate for HCO\(_3\) or oxalate in a Na\(^+\)-independent manner. Although multiple substrates have been proposed for the PAH/dicarboxylate and the sulfate/anion exchanger on the basis of inhibition experiments (188), there is as yet no evidence that these compounds are substrates themselves.

**Organic Anion Transporter Oat1 (PAH/Dicarboxylate Exchanger)**

Various groups have cloned the PAH/dicarboxylate exchanger Oat1 from rat and flounder by expression cloning in *Xenopus laevis* oocytes (165, 175, 190, 204). Furthermore, rat Oat1 sequences have enabled cloning of the human ortholog OAT1 (86% identity to Oat1) (58, 104, 149, 151). Expression of rat Oat1 in *X. laevis* oocytes and human OAT1 in HeLa cells results in uptake of PAH, which is trans-stimulated by glutarate and cis-inhibited by glutarate, \(\alpha\)-ketoglutarate, probenecid, and fluorescein (165, 175, 190, 204). These transport characteristics correspond well to those established for PAH in basolateral membrane vesicles (146). The localization to the basolateral membrane of proximal tubules further supports that OAT1 and Oat1 represent the classical basolateral PAH/dicarboxylate exchanger (58, 185). Oat1 has a broad substrate specificity, and Oat1-mediated PAH transport is inhibited by various nonsteroidal anti-inflammatory drugs, confirming previous results in in situ perfused rat proximal tubules where PAH was used as a substrate (3, 165, 186, 188). The substrate specificity of human OAT1 is narrower, because, unlike rat Oat1 (104), it does not transport methotrexate or prostaglandin E\(_2\). Transport of PAH by both Oat1 and OAT1 is inhibited by phorbol 12-myristate 13-acetate, and this inhibition is reversed by staurosporin, indicating that organic anion uptake is negatively correlated with protein kinase C (PKC) activity (104, 190). These results are in agreement with previous reports on PKC-mediated inhibition of organic anion uptake into the opossum kidney cell line OK and proximal tubules of rabbit, killifish, and flounder by using PAH, fluorescein, or dichlorophenoxyacetate as a substrate (45, 53, 120, 177). Because the Na\(^+\)-K\(^+\)-ATPase, the putative basolateral Na\(^+\)/dicarboxylate exchanger SDCT2, as well as Oat1/OAT1 have multiple PKC phosphorylation sites, it remains to be established whether PKC controls basolateral organic anion transport directly or indirectly.

**Oat2 and Oat3/OAT3**

Functional expression in *X. laevis* oocytes of a putative rat liver transporter, initially designated as NLT, revealed uptake of \(\alpha\)-ketoglutarate, methotrexate, prostaglandin E\(_2\), acetylsalicylate, and PAH similar to rat Oat1 (164, 171). Because rat NLT also shows the highest identity to rat Oat1 (42%), NLT has been renamed Oat2 (164). In addition to expression in liver, Oat2 is also expressed in kidney. Kusuhara et al. (87) have cloned a novel transporter (Oat3), which shares an identity of 49 and 39% with Oat1 and Oat2, respectively. Oat3 is expressed in brain, kidney, liver, and eye and also mediates uptake of PAH and other organic anions. In contrast to Oat1, uptake of organic anions mediated by Oat2 and Oat3 is independent of Na\(^+\) and glutarate (3, 87, 164). This indicates that the presence of a concentration gradient is sufficient for Oat2 and Oat3 to enable organic anion transport. It is at present not clear whether Oat2 and Oat3 mediate efflux and/or reabsorption of organic anions because membrane localization and nephron distribution are not yet established.

Race et al. (149) recently reported the cloning of OAT1 and a kidney-specific homolog called OAT3 (84% identity to Oat3). Expression of OAT3 in *X. laevis* oocytes, however, did not result in uptake of PAH (149), suggesting that OAT3 is not the human ortholog of rat Oat3. Brady et al. (12) have cloned a murine gene encoding a putative transporter (Roc1) with highest...
identity to Oat3 (92%) and OAT3 (83%). Expression of murine Oat3/Roc7 is abundant in kidneys of wild-type mice but markedly reduced in kidneys of mice homozygous for the recessive osteosclerosis (oc) mutation (12). Both the oc mutation as well as the murine oat3/roc7 gene have been mapped to chromosome 19, although no mutations have yet been identified in the oat3/roc7 gene of oc mice (12). Similarly, the OAT3 gene has been mapped closely to the region where human recessive osteopetrosis, a disease with a similar pathophysiology as osteosclerosis, has been mapped (12, 55). Further studies are required to elucidate the relationship between these transporters and the phenotype of osteosclerosis and osteopetrosis.

**Sulfate/Anion Exchanger Sat1**

Expression cloning by using *X. laevis* oocytes has identified a sulfate/anion exchanger (Sat1) from rat kidney and liver (8, 72). In the kidney, Sat1 is located at the basolateral membrane of proximal tubules (72). Expression of Sat1 in SF9 cells results in uptake of sulfate and oxalate, which is cis-inhibited by oxalate and sulfate, respectively (72). In addition, DIDS but not succinate inhibits Sat1-mediated sulfate uptake (8). These transport characteristics are in close agreement with those found in proximal tubule basolateral membrane vesicles (52, 85, 147). The cloning of Sat1 enables one to answer the question whether the compounds proposed as substrates on the basis of earlier inhibition studies are indeed transported by Sat1.

**MRP1**

MRP1 is a member of the large family of ATP-binding cassette proteins and functions as an ATP-dependent transporter of anionic conjugates, such as leukotriene C₄, S-(dinitrophenyl)-glutathione, estradiol-17β-glucuronide, and etoposide-glucuronide (92, 97, 98, 129). MRP1 is expressed in various tissues, including blood cells (38, 213). Mice homozygous for a disrupted *mrp1* gene (*mrp1*−/−) have an impaired response to an inflammatory stimulus, probably as a consequence of decreased leukotriene C₄ secretion from leukocytes (201). Murine Mrp1 is localized to the basolateral membrane of various epithelial cells, and human MRP1 is routed to the basolateral membrane when expressed in the epithelial cell lines LLC-PK₁ and Madin-Darby canine kidney (MDCK) II (32, 34, 202). In murine kidney, Mrp1 is expressed at the basolateral membrane of cells of Henle’s loop and the cortical collecting duct (143, 203). Although previously suggested (154), Mrp1 does not appear to be expressed in the proximal tubule (143, 203).

Human MRP1 is frequently overexpressed in various multidrug resistant cancer cell lines selected with ca-tionic (chemotherapeutic) drugs (20). Transfection of a human *MRP1* cDNA into drug-sensitive cells results in resistance to various cationic drugs, such as vincreistine, doxorubicin, and etoposide (21, 49, 211). In addition, *mrp1*−/− mice are hypersensitive to such drugs (103, 201, 203). Because administration of etoposide to *mrp1*−/− mice results in polyurea, MRP1 might protect distal parts of the nephron, which are exposed to high concentrations of drugs as a result of water reabsorption (203).

**MRP3, MRP4, MRP5, and MRP6**

Searching of the GenBank database EST sequences enabled identification of four additional human MRP-like genes alongside *MRP1* and *MRP2* (see apical transport systems). Expression of *MRP3* (5, 77, 81, 82, 187), *MRP4* (82, 163), *MRP5* (5, 82, 117), and *MRP6* (83) mRNA has been found in various tissues, including kidney. However, Lee et al. (89) did not find expression of *MRP4* in the kidney.

The subcellular localization and nephron distribution of these novel MRPs in the kidney is at present unknown. However, immunohistochemistry on liver sections has demonstrated the presence of human MRP3 at the basolateral membrane of intrahepatic bile-duct epithelial cells (cholangiocytes) and hepatocytes, whereas rat Mrp6 has been located specifically to the hepatocyte lateral membrane (81, 84, 106). Similarly, human MRP5 is confined to the basolateral membrane of various epithelial cells (202). Transport characteristics of several of these novel MRPs have been reported recently. MDCKII cells overexpressing human MRP3 show increased efflux of S-(dinitrophenyl)-glutathione across the basolateral membrane compared with the parental cells (84). Furthermore, MRP3 confers resistance to the chemotherapeutic drugs etoposide, teniposide, and methotrexate (84). In the cell line HepG2, endogenously expressed *MRP3* mRNA is induced by phenobarbital (77). Membrane vesicles from LLC-PK₁ cells transfected with a rat *mrp3* cDNA exhibit ATP-dependent uptake of estradiol-17β-glucuronide and E3040-glucuronide, but not leukotriene C₄ or S-(dinitrophenyl)-glutathione (57). In a T-lymphoid cell line, overexpression of human *MRP4* mRNA and MRP4 protein was found to be correlated with enhanced ATP-dependent efflux of nucleoside monophosphate analogs (163). Substrate specificity of MRP5 has been investigated by expression of a conjugate to green fluorescent protein in HEK-293 cells (117). MRP5-green fluorescent protein-expressing cells preloaded with various fluorescent organic anions show a reduced cellular level of fluorochrome compared with the parental cells (117). In a recent report, rat Mrp6 was shown to mediate ATP-dependent transport of the anionic cyclopentapeptide endothelin antagonist BQ-123 (106). Further studies are required to determine the transport characteristics of these MRPs and to define their role in the kidney.

**INTRACELLULAR DISPOSITION OF ANIONIC DRUGS**

Anionic drugs accumulate in proximal tubules as a result of secretory transport, possibly in combination with reabsorption. The degree of accumulation is de-
formed into cysteine conjugates and mercapturic acid by γ-glutamyltranspeptidase and N-acetyltransferase, respectively (22). The enzymes involved in glucuronide, sulfate, or GSH conjugation are not uniformly distributed in the kidney but show the highest activity in the proximal tubule (61, 96, 101, 209). The different enzymes involved in GSH-derived biotransformation reactions (γ-glutamyltranspeptidase and N-acetyltransferase) express a high activity predominantly in S3 segments of the proximal tubule (60). Biotransformation reactions are primarily detoxification mechanisms, although toxic metabolites may be produced as well [e.g., acetaminophen (27, 132), halogenated alkanes (132), cisplatin (25, 167), cyclosporin A (6), ochratoxin A (28, 46)].

**APICAL TRANSPORT SYSTEMS**

Many of the transport systems for organic anions in the brush-border membrane of the proximal tubule have initially been characterized in membrane vesicle studies (146). Small organic anions, such as PAH and fluorescein, are excreted via a facilitated transporter driven by the potential difference of about −70 mV from cell to lumen. In addition, an anion exchange mechanism has been identified in some species, such as dog, rat, and human, but not in rabbit. Neither of these efflux systems has been cloned. On the basis of inhibition experiments in in situ perfused proximal tubules, Ulrich and Rumrich (189) suggested multiple substrates for these organic anion transporters. Studies with intact killifish proximal tubules have indicated two efflux pathways for organic anions (107, 108, 122). One pathway mediates Na+-dependent efflux of small organic anions, independent of the membrane potential (108, 122). Bulky organic anions, such as fluorescein-methotrexate and lucifer yellow, are excreted via an energy-dependent efflux system, which is insensitive to PAH and depletion of Na+ but inhibited by leukotriene C4 and S-(dinitrophenyl)-glutathione (107, 108). A likely candidate for this transport system is the apical ATP-dependent anionic conjugate transporter Mrp2. Apart from efflux mechanisms, the brush-border membrane also contains transport systems for reabsorption of compounds from primary urine. Small anionic peptides are actively taken up via H+-peptide cotransporters by coupling to the H+ gradient (26). In addition, anionic nucleosides are actively taken up by Na+-nucleoside cotransporters by coupling to the Na+ gradient (39, 50, 203).

**MRP2**

The multidrug resistance protein 2 (MRP2) is an ATP-dependent organic anion transporter with highest identity to MRP1 (48%) and MRP3 (46%). MRP2, also described as the canalicular multispecific organic anion transporter, is expressed at the apical membrane of hepatocytes (14, 139), renal proximal tubules (162), and small intestinal villi (191). In liver, MRPl2 plays an important role in the biliary excretion of multiple conjugated and unconjugated organic anions across the canalicular (apical) membrane. Defective function of
Mrp2, as observed in the two mutant rat strains EHBR and TR /GY, results in hyperbilirubinemia (14, 65, 139). Furthermore, in patients with Dubin-Johnson syndrome, a rare autosomal recessive liver disorder with a similar phenotype as the mutant rats, mutations have been identified in the MRP2 gene (73, 140, 196).

The transport characteristics of Mrp2 have been investigated extensively in comparative studies with wild-type and Mrp2-deficient rats by using perfused liver and isolated canalicular membrane vesicles or hepatocytes (135, 174). In contrast to liver canalicular membrane vesicles, membrane vesicles from the proximal tubule brush-border membrane are unsuitable for characterizing Mrp2-mediated transport. Because these vesicles are exclusively oriented rightside-out, the ATP-binding site is inaccessible for extravesicular ATP (51). Studies with killifish renal proximal tubules have indicated the presence of an MRP-like transporter involved in the energy-dependent and leukotriene C₄-sensitive efflux of fluorescein-methotrexate and lucifer yellow (107, 108). Immunohistochemistry with a polyclonal antibody raised against rabbit Mrp2 has located a killifish ortholog to the brush-border membrane, which further supports this hypothesis (110). Furthermore, studies with membrane vesicles from Mrp2-expressing Sf9 cells have identified PAH as an Mrp2 substrate, suggesting that Mrp2 might be involved in renal clearance of PAH (193). Functional evidence exists for the presence of apical transporters other than Mrp2 involved in renal excretion of organic anions. For example, efflux of lucifer yellow from killifish proximal tubules is only partly inhibited by the Mrp2 substrates leukotriene C₄ and S-(dinitrophenyl)-glutathione (107). Also, renal clearance of the Mrp2 substrates α-naphthyl-β-D-glucuronide (29), E3040-glucuronide (178), cephramide (130), the quinolone HSR-903 (131), and lucifer yellow (Masereeuw R and Russel FGM, unpublished observations) is not impaired in Mrp2-deficient rats.

Like MRP1 and MRP3, human MRp2 not only transports anionic conjugates but also confers resistance to various cationic chemotherapeutic drugs (23, 80). Studies with membrane vesicles from cells expressing human MRP1 have indicated that uptake of cationic drugs, such as vincristine, daunorubicin, and aflatoxin B₁, only occurs in the presence of physiological concentrations of GSH (68, 98–100). A similar GSH dependency of ATP-dependent vinblastine transport has been shown for rabbit Mrp2 (192). This explains the finding that MRP1- and MRP2-mediated drug resistance is reversed by an inhibitor of GSH synthesis (23, 195, 212). For human MRP1, the mechanism involved in GSH-stimulated ATP-dependent transport of vincristine has been identified as a GSH-vincristine cotransport mechanism (99). Daunorubicin and etoposide, unlike vincristine, did not stimulate GSH transport, indicating the involvement of a mechanism different from cotransport (99). Recent studies have shown that MRp2, like MRP1 (141, 212) and MRP5 (202) but not MRP3 (84), transports GSH itself (141). Efflux of GSH from MDCKII cells overexpressing MRp2 is sensitive to depletion of ATP (141). However, studies with membrane vesicles have indicated that rabbit Mrp2 is permeable for GSH (192).

Regulation of Mrp2-mediated transport has been investigated in isolated rat hepatocytes and killifish renal proximal tubules; however, results in these tissues are at variance. Both cAMP and PKC stimulated Mrp2-mediated efflux of an anionic conjugate across the hepatocyte apical membrane (155, 156). At least for the effect of cAMP, this efflux has been shown to be a result of stimulated sorting of Mrp2-containing vesicles to the apical membrane (156). In the killifish proximal tubule, efflux of fluorescein-methotrexate is negatively correlated with PKC activity (110). Activation of the signal transduction pathway occurs by binding of endothelin-1 to the B-type receptor (110). Because endothelins are involved in many processes in the kidney, this suggests a specific regulatory pathway for renal Mrp2.

Organic Anion Transport Polypeptides Oatp1 and Oatp3

The organic anion-transporting polypeptide 1 (Oatp1) is a Na⁺- and ATP-independent transporter originally cloned from rat liver (67). Oatp1 is localized to the basolateral membrane of hepatocytes, where it plays a major role in uptake of a variety of anionic, neutral, and cationic compounds from the blood (118). In contrast, Oatp1 is located at the apical membrane of S3 proximal tubules (7). Studies with transiently transfected HeLa cells have indicated that Oatp1 mediates uptake of taurocholate in exchange for HCO₃⁻ (160). In addition, uptake of taurocholate by Oatp1 expressed in X. laevis oocytes is accompanied by efflux of GSH (93). As an anion/GSH exchanger, Oatp1 might mediate reabsorption of organic anions rather than efflux in the kidney. In this respect, reabsorption of ochratoxin A in proximal tubules is inhibited by bromosulfophthalein, which suggests the involvement of Oatp1 as both compounds have been identified as substrates (24, 31). On the other hand, because Oatp1 transports various anionic conjugates it might function as an efflux transporter, representing the transport mechanism maintained in kidneys of Mrp2-deficient rats. Abe et al. (1) reported the cloning of an Oatp1 homolog, designated Oatp3 (80 and 82% identity to Oatp1 and Oatp2, respectively). Oatp3 is highly expressed in kidney and mediates transport of thyroid hormones and taurocholate, like Oatp1 and Oatp2 (1). However, no data are yet available on the nephron distribution and membrane localization of Oatp3.

Organic Anion Transporters Oat-k1 and Oat-k2

Oat-k1 and Oat-k2 are kidney-specific Na⁺- and ATP-independent organic anion transporters with highest identity to Oatp1 (72 and 65%, respectively) (112, 157). Both transporters are confined to the brush-border membrane of proximal tubular cells (112, 116). Oat-k1 and Oat-k2 mediate transport of methotrexate...
and folate, whereas Oat-k2 also transports prostaglan-
din E₂ and taurocholate (112, 157). Additional studies are
required to elucidate whether Oat-k1 and Oat-k2
mediate efflux or reabsorption of organic anions. How-
ever, Oat-k1 expressed in MDCK cells transports
methotrexate across the apical membrane in both di-
rections (114). Similarly, Oat-k2 transports tauro-
cholate bidirectionally across the apical membrane
on expression in MDCK cells (112). In a recent study,
Oat-k1 was proposed as the ochratoxin A reabsorption
pathway in proximal tubules on the basis of the inhib-
itory effect of bromosulfophthalein (24). Although bro-
mosulfophthalein indeed inhibits Oat-k1-mediated
transport (114, 157), it also inhibits Oat-k2-mediated
transport (112). Furthermore, there is no evidence to
suggest that ochratoxin A is a substrate of either of
these transporters. In this respect, various organic
anions (i.e., nonsteroidal anti-inflammatory drugs,
PAH, digoxin, probenecid), bile acid analogs, and ste-
roids are potent inhibitors but not substrates of Oat-k1
and Oat-k2 (112–115, 157). Several of these drugs can
accumulate to high concentrations in the proximal
tubule, suggesting that under these conditions both
transporters are inhibited.

Na⁺-Nucleoside Cotransporters CNT1 and CNT2

The concentrative nucleoside transporters CNT1
and CNT2 are involved in Na⁺-dependent transport of
endogenous nucleosides and various synthetic (ani-
onic) nucleosides, which are of clinical importance for
their use in the treatment of cancer and viral infections
(138). CNT1 (N2 subtype or cit) is selective for pyrimi-
dines, whereas CNT2 (N1 subtype or cif) favors trans-
port of purines, although uridine and adenosine are
transported by both proteins (17, 35, 59, 152, 153, 161,
198, 207, 208). Structural features markedly distin-
guish rat Cnt2 from human CNT2; moreover, Cnt2
transports thymidine in contrast to CNT2 (17, 153,
198). Northern blotting and RT-PCR have identified
expression of human CNT1 and CNT2 mRNA in the
kidney (152, 153, 198). Although their membrane lo-
calization has not been established by immunohis-
tochemistry, Na⁺-nucleoside cotransport into cells
overexpressing CNT1 or CNT2 resembles transport
characteristics found in brush-border membrane vesi-
cles (39, 50, 204). This indicates that CNT1 and CNT2
mediate Na⁺-nucleoside cotransport across the brush-
border membrane into the proximal tubule.

H⁺-Peptide Cotransporters PEPT1 and PEPT2

Peptide transporters are involved in H⁺-dependent
transport of small peptides and various peptide-like
compounds such as anticancer drugs (bestatin, delta-
aminovalin acid), prodrugs (L-dopa-L-Phe, L-Val-
azidothymidine), inhibitors of angiotensin-converting
enzyme (captopril, enalapril), and various anionic
β-lactam antibiotics such as cephalosporins (cepha-
lexin, cepharadine, cefadroxil, cefdinir) and penicillins
(cyclicalin, ampicillin) (26, 90). Two peptide transport-
ers have been cloned, i.e., a low-affinity transporter
from intestine (PEPT1) and a high-affinity transporter
from kidney (PEPT2) (26, 90). Immunohistochemistry
has located PEPT2 to the proximal tubule brush-bor-
der membrane, and characteristics of H⁺-peptide co-
transport into various cell types expressing PEPT2
resemble transport characteristics found in brush-bor-
der membrane vesicles (9, 126, 127, 150, 168, 170, 176,
184). This indicates that PEPT2 mediates H⁺-peptide
cotransport from the primary urine into the proximal
tubule cell (26, 168). PEPT1 has also been located to
the brush-border membrane but is difficult to charac-
terize in membrane vesicles due to its relatively low
expression and the interference of substrates with
PEPT2 (26). However, the angiotensin-converting en-
zeyme inhibitors enalapril and captopril are not trans-
ported by PEPT2, and these drugs have recently been
used to characterize PEPT1-mediated transport in kid-
ney (181).

CONCLUSIONS

The past few years have witnessed great advances in
our understanding of the molecular pharmacology of
renal organic anion transport. Considerable progress
has been made in cloning key proteins involved in
the transport of anionic xenobiotics and metabolites,
and the list of cloned organic anion transporters is steadily
growing. The challenge of future work will be to inte-
grate this information with (patho)physiological, phar-
macological, and toxicological investigations at the cel-
luar and organ level. There is still a large gap in our
knowledge about the relative contribution of individual
transporters to the membrane steps involved in tubu-
lar secretion of specific anionic substrates. More de-
tailed knowledge of the cloned carrier proteins in ex-
pression systems and the availability of specific
antibodies will allow more fundamental insight into
membrane translocation at the molecular level, as well
as participation in overall transepithelial secretion.
This will also facilitate the research on the interaction
of cellular messengers with the carrier proteins and
the way these transporters are synthesized and tar-
geted to specific membrane sites in the normal and
diseased kidney. An important approach to study the
in vivo function of transporters and their mutual in-
teraction is to develop and characterize (multiple) null
mutants of the genes encoding these proteins. Finally,
it has become increasingly clear that intracellular dis-
position is an important step in the active secretion of
anionic xenobiotics. How these processes interact with
the membrane transport mechanisms to produce secre-
cion and at what level and to what extent they are
regulated will be important questions to answer.

A detailed knowledge of the renal mechanisms that
govern intracellular distribution and membrane trans-
port of xenobiotics in the kidney is essential for the
development of clinically useful drugs and will advance
our understanding of the molecular, cellular, and clin-
ical bases of renal drug clearance, drug-drug interac-
tions, drug targeting to the kidney, and xenobiotic-
induced nephropathy.
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