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PERSPECTIVES IN BASIC SCIENCE

Cellular and molecular aspects of drug transport in the kidney

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Cellular and molecular aspects of drug transport in the kidney. The kidney plays an important role in the elimination of numerous hydrophilic xenobiotics, including drugs, toxins, and endogenous compounds. It has developed high-capacity transport systems to prevent urinary loss of filtered nutrients, as well as electrolytes, and simultaneously to facilitate tubular secretion of a wide range of organic ions. Transport systems for organic anions and cations are primarily involved in the secretion of drugs in renal tubules. The identification and characterization of organic anion and cation transporters have been progressing at the molecular level. To date, many members of the organic anion transporter (OAT), organic cation transporter (OCT), and organic anion-transporting polypeptide (oatp) gene families have been found to mediate the transport of diverse organic anions and cations. It has also been suggested that ATP-dependent primary active transporters such as MDR1/P-glycoprotein and the multidrug resistance-associated protein (MRP) gene family function as efflux pumps of renal tubular cells for more hydrophobic molecules and anionic conjugates. Tubular reabsorption of peptide-like drugs such as β-lactam antibiotics across the brush-border membranes appears to be mediated by two distinct H^+/peptide cotransporters: PEPT1 and PEPT2. Renal disposition of drugs is the consequence of interaction and/or transport via these diverse secretory and absorptive transporters in renal tubules. Studies of the functional characteristics, such as substrate specificity and transport mechanisms, and of the localization of cloned drug transporters could provide information regarding the cellular network involved in renal handling of drugs. Detailed information concerning molecular and cellular aspects of drug transporters expressed in the kidney has facilitated studies of the mechanisms underlying renal disposition as well as transporter-mediated drug interactions.

The kidney, as well as the liver, plays a pivotal role in the elimination of numerous potentially toxic xenobiotics, including drugs, toxins, and endogenous metabolites. Renal elimination of drugs involves glomerular filtration, tubular secretion, and tubular reabsorption. Of these, the secretion and reabsorption of drugs in renal tubules are essentially saturable processes, as plasma membrane transport proteins mediate vectorial transepithelial transport. The kidney has developed high-capacity transport systems to prevent urinary loss of filtered nutrients such as D-glucose, amino acids, and oligopeptides, as well as inorganic ions, and simultaneously to facilitate tubular secretion of a variety of xenobiotics that escape hepatic extraction processes.

The transport systems responsible for renal tubular secretion of drugs have been classified as either organic anion or cation transport systems based on their preferential substrate selectivity [1, 2]. The secretory transport process is performed effectively by two distinct classes of transporters: one localized at the basolateral membranes to mediate cellular uptake of substrates from blood and the other at the brush-border membranes to mediate exit of cellular substrates into the tubular lumen [3–5]. Over the last several years, considerable progress has been made regarding the identification and characterization of organic anion and cation transporters at the molecular level [6–9]. In addition to these organic ion transporters, the adenosine 5′-triphosphate (ATP)-dependent primary active transporters such as P-glycoprotein [10, 11] and the multidrug resistance-associated protein (MRP) family have been suggested to function as the drug efflux pumps in renal tubules [12, 13].

Renal reabsorption of some classes of drugs is mediated by nutrient transporters in proximal tubules. Several peptide-like drugs such as oral β-lactam antibiotics are known to undergo both secretion and reabsorption in renal tubules [14]. Organic anion and cation transporters have been suggested to mediate the secretory transport of these antibiotics. The reabsorption of most peptide-like drugs from the glomerular filtrate is mediated by oligopeptide transporters localized at the brush-border membranes of proximal tubular cells, influencing their pharmacokinetic profiles and therapeutic efficacy [14]. Therefore, renal handling of most charged drugs is the result of the interaction and transport processes through secretory organic ion transporters and, in certain cases, through the reabsorptive nutrient transporters in renal tubular cells. It is imperative to obtain detailed information about "kidney-specific drug transporters" for a better understanding of renal tubular secretion and/or reab-

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sorption of drugs. Such information would be of clinical importance for predicting interactions between drugs and/or their metabolites with renal transporters, which may result in competition at a tubular secretion or reabsorption site and be accompanied by significant adverse effects.

This review deals with the recent advances in cellular and molecular studies regarding identification and comparative characterization of renal drug transporters. An insight into their pharmacological and toxicological characteristics is also given, as well as a brief historical background.

FUNCTIONAL CHARACTERISTICS OF ORGANIC ANION TRANSPORT SYSTEMS

More than 60 years ago, Smith, Goldring, and Chassis reported that the anionic dye phenol red was eliminated from renal plasma in a single pass through the kidney [15]. Since then, the renal organic anion transport system has been extensively studied, and this secretory system appears to mediate renal excretion of a wide range of small foreign compounds, including drugs, their metabolites, and diverse xenobiotics. Thus, the organic anion transport system plays a critical role in protecting against the potential toxic effects of anionic compounds by mediating their excretion into urine.

The secretion of organic anions in the renal tubules is mediated by the concerted function of two distinct transport steps at the peritubular basolateral membranes and luminal brush-border membranes of the tubular cells (Fig. 1) [3–7]. The energy-dependent uphill uptake is required for the basolateral entry step of the negatively charged anions, as they must be translocated against an electrical potential barrier across the basolateral membranes. Shimada, Moewes, and Burckhardt reported that uptake of para-aminohippurate (PAH), a marker substrate for the renal organic anion transport system in the isolated basolateral membrane vesicles, was markedly enhanced in the presence of an outward α-ketoglutarate gradient [16]. This finding suggested that dicarboxylates are the physiological counterions for the uphill transport of organic anions. The outward α-ketoglutarate gradient is sustained not only by intracellular metabolic dicarboxylate generation, but also by the Na^+/α-ketoglutarate cotransport system driven by the inward Na^+ gradient [17], which is established by Na^+,K^+-ATPase. Therefore, the basolateral transport of organic anions appears to be driven by indirect coupling to the Na^+ gradient through Na^+/α-ketoglutarate cotransport and organic anion/dicarboxylate exchange (Fig. 1, #1) [18].

In rat renal brush-border membrane vesicles, the uptake of PAH was stimulated by an inside-positive membrane potential created by K^+ and valinomycin [19]. In addition, PAH uptake was stimulated by PAH/PAH or

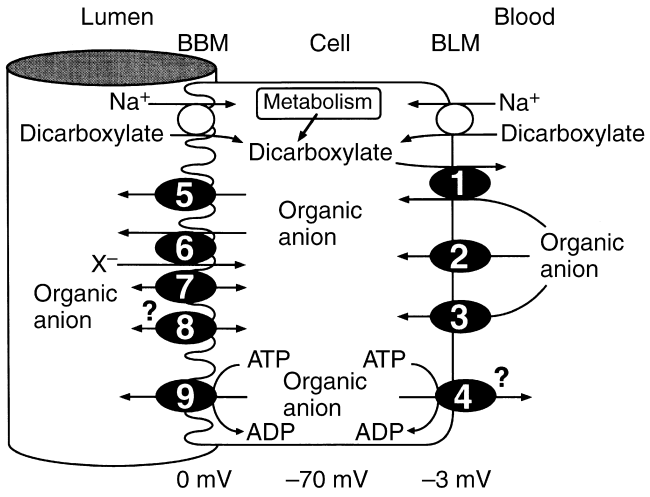

Fig. 1. Mechanisms of organic anion transport in renal tubular cells. Cellular uptake of organic anions across basolateral membranes (BLM) is mediated by OAT1 (1), which is an organic anion/dicarboxylate exchanger, and by OAT2 (2) and OAT3 (3). Anionic drug conjugates with glutathione may be extruded from cells into blood by MRP1 (4). Exit of cellular organic anions across brush-border membranes (BBM) is mediated by unidentified transmembrane potential-dependent organic anion transporter (5) and organic anion/anion (X^-) exchanger (6). Bidirectional transport of hydrophobic anions such as methotrexate and folic acid in the brush-border membranes is mediated by OAT-K1 (7). OAT-K2 (8) may also participate in tubular reabsorption and/or secretion of hydrophobic anions such as bile acids, methotrexate, and prostaglandin E_2. MRP2/cMOAT (9) may contribute to tubular secretion of anionic conjugates of hydrophobic compounds.

PAH/Cl^- exchange, and the PAH/PAH exchange was insensitive to the membrane potential. The potential-stimulated PAH uptake was more sensitive to anionic drugs such as furosemide and 4,4′-diisothiocyano-2,2′-disulfonic stilbene than PAH/PAH exchange [20]. These findings suggest that PAH is transported by two distinct transport systems in rat renal brush-border membranes, that is, a potential-sensitive transport system (Fig. 1, #5) and an anion exchanger (Fig. 1, #6). The anion exchanger is found to be expressed in urate-reabsorbing species such as the rat and the dog, and accepts various organic and inorganic anions (including PAH, urate, Cl^-, Br^-, HCO_3^-, and OH^-) as substrates [21, 22]. In addition to these proposed organic anion transport systems, functional studies using isolated perfused renal tubules have demonstrated the participation of multiple transport proteins in secretion of organic anions at the brush border membranes (discussed later in this article) [7].

IDENTIFICATION OF ORGANIC ANION TRANSPORTERS

Oat family

As the first step toward the molecular characterization of organic anion transport (OAT) proteins through expression cloning, the functional expression of both fum-

arate- and lithium-sensitive glutarate and probenecid-sensitive PAH transporter were investigated in *Xenopus* oocytes injected with rat kidney poly(A)$^+$ RNA [23]. Expression of size-fractionated mRNA indicated that the active species, with respect to PAH transport activities, was in the range of 1.8 to 3.5 kb. In 1997, Sekine et al [24] and Sweet, Wolff, and Pritchard [25] successfully isolated a 2.2 kb cDNA clone from rat kidney encoding the PAH/dicarboxylate exchanger protein by expression cloning with oocytes. This was designated as OAT1. Rat (r) OAT1 is comprised of 551 amino acid proteins with 12 putative membrane-spanning domains. Wolff et al also isolated the cDNA clone from winter flounder coding for the PAH/dicarboxylate exchange, designated as fOAT1, which was 2.8 kb in length and encoded a protein of 562 amino acids [26]. When expressed in oocytes, both rat and flounder OAT1 mediated Na^+-independent PAH uptake with apparent Km values ranging from 14.3 to 70 μmol/L [24–26]. The uptake rate of PAH was markedly enhanced in the presence of an outward gradient of dicarboxylate, indicating that OAT1 functions as an organic anion/dicarboxylate exchanger [24–26]. rOAT1 had a wide substrate specificity for endogenous anions such as cyclic nucleotides, prostaglandins, uric acid, and structurally diverse drugs such as β-lactam antibiotics, methotrexate, and nonsteroidal anti-inflammatory drugs (NSAIDs) [27, 28]. In addition, it was reported that rOAT1 mediated transport of ochratoxin A, a potent nephrotoxin, suggesting that accumulation of the toxin via rOAT1 in proximal tubules may be the primary event in the development of ochratoxin A-induced nephrotoxicity [29]. Immunohistochemical analysis suggested that rOAT1 is exclusively expressed in the S2 segments of proximal tubules in the kidney and is localized to the basolateral membranes of these segments [30].

Two cDNAs, hOAT1-1 encoding 556 amino acid proteins [31] and hOAT1-2 (PAHT, hROAT1) encoding 550 amino acid proteins [31–34] were identified in the human kidney. hOAT1-1 as well as rOAT1 appeared to be the PAH/dicarboxylate exchanger localized to the basolateral membranes of proximal tubule and multispecific for various xenobiotics and endogenous substances [31]. In contrast, PAHT was suggested to show a narrow substrate specificity, since prostaglandins and methotrexate were not transported [33]. There may be differences in substrate specificity between the two hOAT1 isoforms, which could be derived by the alternative-splicing mechanisms.

It has been demonstrated that a cultured renal epithelial cell line derived from opossum kidney (OK) possesses transcellular transport activity for PAH, corresponding to renal tubular secretion [35, 36]. Studies using various organic anions showed that the PAH transport system in the basolateral membranes of OK cells has a similar substrate specificity to that in renal proximal tubules [37]. In addition, efflux of intracellular α-ketoglutarate from the OK cells to the basolateral side medium was stimulated in the presence of PAH, suggesting that the PAH/dicarboxylate exchange system functions in this cell line [38]. Partial sequence analysis by reverse transcription-polymerase chain reaction (RT-PCR) suggested that the opossum homologue of OAT1 was expressed in OK cells (unpublished data). PAH uptake across the basolateral membranes of OK cell monolayers grown on microporous membrane filters was markedly inhibited by a phorbol esters such as phorbol 12-myristate 13-acetate, suggesting that the PAH transport system in OK cells could be under the regulatory control of protein kinase C [39]. Parathyroid hormone was found to inhibit PAH uptake by OK cells through protein kinase C activation and was assumed to be involved in the regulation of the organic anion transporter [39]. Similar inhibitory regulation by protein kinase C as well as substrate specificity has been demonstrated for rOAT1 expressed in oocytes [28]. The physiological implications for this regulation of OAT1 remain to be elucidated.

Other members with significant homology to OAT1 have been identified (Table 1).

Novel liver-specific transport protein (NLT), isolated as a liver-specific transporter from the rat [40], shows a 42% identity with rOAT1. When expressed in oocytes, NLT mediated the uptake of organic anions such as salicylate, acetylsalicylate, prostaglandin E_2, dicarboxylates, and PAH [41]. Expression of NLT mRNA was predominantly detected in the liver and to a lesser extent in the kidney [40, 41]. Thus, it has been postulated that rat NLT should be renamed rOAT2. In contrast to OAT1, NLT/rOAT2 appears not to be an organic anion/dicarboxylate exchanger, and its driving force has not been identified (Fig. 1, #2).

A cDNA encoding another member of the multispecific organic anion transporter family, rOAT3, was isolated from rat brain by the RT-PCR cloning method based on the sequence conserved among rOAT1, rOAT2 and rat organic cation transporter (rOCT1; Fig. 1, #3) [42]. rOAT3 shows 49, 39, and 36% identity with rOAT1, rOAT2, and rOCT1, respectively. rOAT3 mRNA appeared to be expressed in the liver, brain, kidney, and eye [42]. When expressed in oocytes, rOAT3 mediated the uptake of organic anions such as PAH (Km value of 65 μmol/L), ochratoxin A (Km value of 0.74 μmol/L), and estrone sulfate (Km value of 2.3 μmol/L), and the cationic drug cimetidine. rOAT3-mediated uptake of estrone sulfate was inhibited by other anions such as sulfobromophthalein (BSP), probenecid, indocyanine green, bumetanide, piroxicam, furosemide, azidodeoxythymidine, and benzylpenicillin, but not by cationic compounds such as tetraethylammonium (TEA), guanidine, or quinidine [42]. rOAT3 has been suggested to partici-

Table 1. Organic ion transporter family

Transporter	Species	Accession	Chromosome	Tissue distribution	Substrates[a]
OAT1	Human (SLC22A6) Rat Mouse (NKT) Winter flounder	AB009697 AB004559 U52842 Z97028	11q11-q13.1	Kidney≫brain (rat)	PAH, α-KG, cAMP, cGMP, NSAIDs, methotrexate, β-lactam antibiotics, ochratoxin A, uric acid, prostaglandin E_2
OAT2	Human (SLC22A7, NLT) Rat (NLT)	AF097518 L27651		Liver≫kidney (rat)	PAH, α-KG, salicylate, acetylsalicylate, prostaglandin E_2
OAT3	Human (SLC22A8) Rat	AF097491 AB017466	11q11-q12	Liver>kidney>brain (rat)	PAH, ochratoxin A, estrone-3-sulfate, cimetidine
OCTN1	Human (SCL22A4) Rat Mouse	AB007448 AF169831 AF111425	5	Kidney, spleen, bone marrow, etc. ubiquitous (human)	L-carnitine, quinidine, verapamil, TEA
OCTN2	Human (SLC22A5) Rat (CT1, UST1) Mouse	AF057164 AB017260 AF110417	5q31	Kidney, skeletal muscle, placenta, pancreas (human)	L-carnitine, TEA
OCT1	Human (SLC22A1) Rabbit Rat Mouse	U77086 AF015958 X78855 AF010259	6q26	Liver, kidney>small intestine (rat)	TEA, NMN, choline, dopamine, MPP
OCT1A	Rat	U76379			TEA
OCT2	Human (SLC22A2) Pig Rat Mouse	X98333 Y09400 D83044 AJ006036	6q26	Kidney≫brain (rat)	TEA, choline dopamine, MPP, guanidine
OCT3	Human (SLC22A3) Rat Mouse	AF078749 AF055286 AF078750	6q26-q27	Placenta≫small intestine, heart, brain>kidney, lung (rat)	TEA, guanidine
RST	Mouse	AB05451		Kidney	
UST2	Rat	AJ001933		Ubiquitous	

[a] The substrate specificities were examined for each rat clone

pate in the excretion and/or detoxification of endogenous and exogenous organic anions, especially from the brain.

Organic anion-transporting polypeptide family

In 1994, Jacquemin et al isolated a cDNA clone from rat liver coding for the organic anion-transporting polypeptide (oatp1) by expression cloning [43]. The clone encodes 670 amino acids with 10 putative transmembrane domains. The oatp1-mediated Na^+-independent uptake of BSP which was Cl^- dependent in the presence of bovine serum albumin [43]. In addition to BSP, oatp1 also mediated Na^+-independent uptake of conjugated and unconjugated bile acids. Northern blot analysis demonstrated expression of oatp1 mRNA in rat liver, kidney, brain, lung, skeletal muscle, and proximal colon. Immunohistochemical examination of the liver revealed the sinusoidal plasma membrane localization of oatp1 [44]. In rat kidney, oatp1 was detected in renal brush-border membranes in the S3 segment of the proximal tubule of the outer medulla [44]. Differential processing and trafficking of oatp1 in the liver and kidney have been suggested. As oatp1 mRNA is strongly expressed in the kidney, it has been suggested that the transporter participates in the secretion of a variety of organic anions by the kidney. In the HeLa/vaccinia transient expression system, oatp did not mediate transport of PAH, urate, sulfate, or eicosanoids [45]. The oatp1-mediated uptake of BSP was inhibited by corticosterone sulfate, spironolactone, and several other steroids, which could represent high-affinity endogenous oatp1 substrates. Estradiol 17β-D-glucuronide appeared to be one of the preferred substrates of oatp1, suggesting that the transporter may serve as an apical exit pathway for steroids following the conjugated steroids with a strong 17- or 3-position anionic group in tubules [45].

Two cDNAs encoding other members of the oatp gene family, oatp2 [46] and oatp3 [47], were isolated from the rat brain and retina, respectively (Table 2). Oocytes injected with synthetic RNA encoding oatp2 and oatp3 showed an uptake of taurocholate, thyroxin, and triiodothyronine in a saturable manner. In addition, the oatp2-expressing oocytes stimulated the uptake of cardiac glycosides such as digoxin and ouabain. The oatp2 mRNA was found to be widely expressed in central nervous system neuroneal cells as well as in the retina and liver. In contrast, oatp3 mRNA was expressed in the kidney and moderately expressed in the retina. It has been suggested that oatp2 and oatp3 are multifunctional trans-

Table 2. Organic anion transporting polypeptide family

Transporter	Species	Accession	Chromosome	Tissue distribution	Substrates[a]
oatp1	Rat	L19031		Liver>kidney	BSP, ouabain, taurocholic acid, cholic acid, 17β-estradiol glucuronide, leukotriene C_4, DNP-SG
oatp2	Rat	U88036		Liver>brain>retina	Digoxin, ouabain, taurocholic acid, cholic acid, 17β-estradiol glucuronide, thyroxine, 3,5,3′-triiodo-L-thyronine
oatp3	Rat	AF041105		Kidney>retina, liver	Taurocholic acid, thyroxine, 3,5,3′-triiodo-L-thyronine
OATP	Human (SLC21A3)	U21943	12	Brain	BSP, taurocholic acid, cholic acid, glycocholic acid, taurochenodeoxycholic acid, tauroursodeoxycholic acid
OAT-K1	Rat	D79981		Kidney	Methotrexate, folic acid
OAT-K2	Rat	AB012662		Kidney	Methotrexate, folic acid, taurocholic acid, prostaglandin E_2
PGT	Human Rat (matrin F/G)	U70867 M64862	3q21	Ubiquitous (human)	Prostanoids

[a] The substrate specificities were examined for each rat clone except *OATP*

porters in the brain, retina, liver, and kidney. The membrane localization of both transporters has not yet been identified.

In 1996, a cDNA encoding a member of the oatp gene family, designated as OAT-K1, was isolated in our laboratory (Table 2) [48]. Rat OAT-K1 is comprised of 669 amino acids and shows 72% identity with rat oatp1. Northern hybridization indicated that OAT-K1 mRNA of 2.8 kb was expressed exclusively in rat kidney. In LLC-PK_1 cells stably transfected with OAT-K1 cDNA, the transporter protein was localized in the basolateral membranes and mediated Na^+-independent uptake of methotrexate (Km value of 1 μmol/L), folate, but not of PAH, taurocholate, prostaglandin E_2, or leukotriene C_4 [48]. By RT-PCR detection, OAT-K1 mRNA was found predominantly in superficial and juxtamedullary proximal straight tubules [49]. Western blotting with antirat OAT-K1 antibodies revealed that a transporter protein with an apparent molecular mass of 40 kD (calculated molecular mass of 74 kD) was expressed exclusively in rat kidney brush-border membranes (Fig. 1, #7) [49]. When transfected into Madin-Darby canine kidney (MDCK) cells, OAT-K1 mediated bidirectional transport of methotrexate at the apical membranes, but not at the basolateral membranes [50]. The protein with an apparent molecular mass of 50 kD was detected in the plasma membrane fractions of MDCK cells transfected with OAT-K1 by Western blotting. In contrast, OAT-K1 protein of about 70 kD was found in the basolateral membranes of the transfected LLC-PK_1 cells [49]. Therefore, we suggested that the proteolytic processing and/or excision of OAT-K1 found in renal tubular cells would be retained in MDCK cells, but not in LLC-PK_1 cells. Moreover, we found that the mechanisms of the membrane sorting of OAT-K1 are different between MDCK and LLC-PK_1 cells. In the OAT-K1-transfected cells, NSAIDs such as indomethacin and ketoprofen inhibited transport of methotrexate in a competitive manner [51]. Other NSAIDs, including ibuprofen, flufenamate and phenylbutazone, but not salicylate, showed potent inhibitory effects on the OAT-K1–mediated uptake of methotrexate. These findings suggested that OAT-K1 could be one of the sites of interaction for methotrexate and NSAIDs in the kidney.

Another kidney-specific isoform of the oatp family, designated as OAT-K2 [52], was isolated from rat kidney. OAT-K2 is comprised of 498 amino acids and shows 91% identity with rat OAT-K1. OAT-K2 mRNA was detected in proximal convoluted and straight tubules and cortical collecting ducts. In contrast to OAT-K1, OAT-K2 mediated the uptake of various hydrophobic anions such as taurocholate, methotrexate, folate, and prostaglandin E_2. When transfected into MDCK cells, OAT-K2 was localized functionally to the apical membranes and transported taurocholate with a Km value of 10 μmol/L. Several organic anions, bile acids, cardiac glycosides, and steroids showed potent inhibitory effects on OAT-K2–mediated uptake of taurocholate. OAT-K2 may participate in reabsorption and/or secretion of hydrophobic anions in the kidney (Fig. 1, #7).

Multidrug resistance-associated protein family

MRP1, identified from a human multidrug-resistant lung cancer cell line as a glutathione S-conjugate (GS-X) pump [53] able to transport anionic drug conjugates as well as unmodified anticancer drugs out of the cell, also plays a role in detoxification in tissues including the liver, lung, and kidney (Fig. 1, #4) [54, 55]. MRP1 is an integral membrane glycoprotein, belonging to the ATP-binding cassette (ABC) transporter superfamily [54]. The most extensively studied member of the MRP family is MRP2/cMOAT, the canalicular multispecific organic anion transporter, which was found to be defective in mutant rats, such as Eisai hyperbilirubinemic (EHBR) [56, 57]

Table 3. ABC transporter family

Transporter	Species	Accession	Chromosome	Tissue distribution	Substrates[a]
Pgp	Human (MDR1, ABCB1) Rat (mdr1b) Mouse (mdr1a) Mouse (mdr1b)	M14758 L15079 M33581 J03398	7q21	Liver, small intestine, kidney, brain, adrenal gland (human)	Hydrophobic (cationic) compounds, anticancer agents, digoxin, immunosuppressants, steroids
MRP1	Humans (GS-X, ABCC1) Mouse	L05628 AF022908	16p13.1	Liver, brain, kidney (human)	Anticancer agents, anionic conjugates with glutathione, sulfate or glucuronide
MRP2	Human (cMOAT, ABCC2) Rat (cMOAT)	U49248 AB017446	10q24	Liver, kidney (rat)	Organic anions, anionic conjugates with glutathione, sulfate or glucuronide, bilirubin glucuronide
MRP3	Human (cMOAT2, ABCC3) Rat (MLP2)	AB010887 AB010467	17q21.3	Liver, small intestine (human)	Anionic conjugates
MRP6	Human (ABCC6) Rat (MLP1) Mouse	AF07662 U73038 AB028737	16p13.1		

[a] The substrate specificities were examined for each human clone

and TR$^-$ [58] rats. MRP2/cMOAT shares 49% amino acid identity with MRP1. The absence of MRP2/cMOAT in humans results in the Dubin–Johnson syndrome [59]. Most substrates for MRP1 and MRP2/cMOAT are anionic conjugates of hydrophobic substances such as the glutathione S-conjugate leukotriene C_4 and the glucuronide conjugate of bilirubin. The Km value (1.0 μmol/L) of human MRP2/cMOAT for leukotriene C_4 was tenfold higher than that of MRP1 (0.1 μmol/L) [54]. There are kinetic differences between the two MRP isoforms, but no substrates or inhibitors that are selective for either MRP1 or MRP2/cMOAT have yet been found. MRP2/cMOAT was identified in the brush border membranes of segments S1, S2, and S3 of rat kidney proximal tubules by immunohistochemical analysis [12]. MRP2/cMOAT in the kidney has been suggested to contribute to cellular detoxification and to the secretion of endogenous and xenobiotic anionic compounds, most of which are conjugates, from the blood into urine (Fig. 1, #9).

Other members of the MRP gene family, MRP3 and MRP6, were found to be expressed in the kidney (Table 3). MRP3 was found in human liver, colon, pancreas, but was less abundant in the kidney [60]. MRP3 was suggested to be the basolateral MRP isoform, which mediates the cellular extrusion of glucuronide conjugates, and to be up-regulated when the canalicular secretion of anionic conjugates by MRP2/cMOAT in the liver is impaired [61]. In MDCK cells transfected with MRP3 cDNA, MRP3 was localized to the basolateral membranes and mediated efflux of the organic anion S-(2,4-dinitrophenyl-)glutathione into the basolateral side of the monolayer [62]. The most recently discovered member of the family, MRP6, was found to be highly expressed in the liver and kidney and weakly expressed in a few other tissues [13]. The intrarenal distribution, membrane localization, and substrate selectivity of MRP6 have not been elucidated.

FUNCTIONAL CHARACTERISTICS OF ORGANIC CATION TRANSPORT SYSTEMS

In the kidney, organic cation transport systems play physiological and pharmacological roles in the reabsorption and/or excretion of endogenous organic cations such as guanidine, choline, N^1-methylnicotinamide (NMN), bioactive monoamines (dopamine, epinephrine, and histamine), cationic drugs (TEA, cimetidine, procainamide, and quinidine), and cationic toxins. In this way, the homeostasis of these diverse cations is maintained. Organic cation transport activity has been found primarily in renal proximal tubules, but has also been detected in distal tubules and collecting ducts [63–65]. Functional measurements of organic cation transport system were examined employing isolated renal tubules [66–68], stop-flow microperfusion of proximal tubule [69], and plasma membrane vesicles isolated from renal proximal tubules [70–83]. These studies suggested that secretion of cationic substances in renal tubules is operated effectively by the cooperative function of two distinct organic cation transporters: one facilitated by the transmembrane electrical potential difference in the basolateral membranes and the other driven by the transmembrane H^+ gradient in the brush border membranes (Fig. 2, #4). LLC-PK_1 cells derived from porcine kidney have been demonstrated to mediate unidirectional transcellular transport of TEA from the basolateral medium to the apical medium, corresponding to renal tubular secretion [84]. We found that LLC-PK_1 cells are useful for studying mechanisms of cationic xenobiotics and drug interactions mediated by organic cation transporters in vitro [85–87].

In 1979, Kinsella et al reported initial studies of NMN uptake by the basolateral and brush-border membrane vesicles from dog kidney, suggesting the existence of distinct transport systems at each membrane [76]. We reported that the valinomycin- and K^+-induced trans-

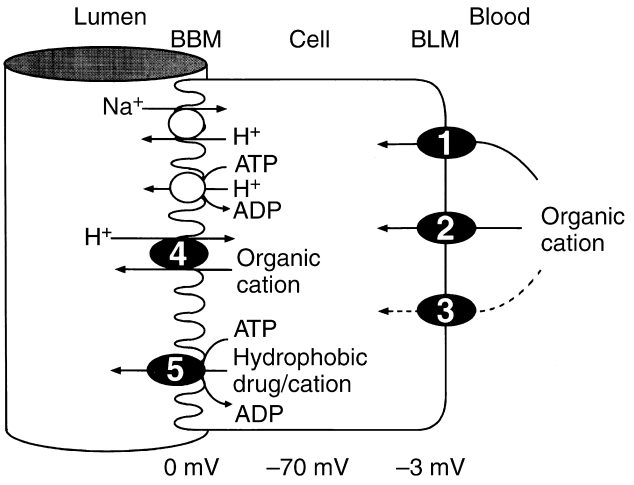

Fig. 2. Mechanisms of organic cation transport in renal tubular cells. Cellular uptake of organic cations across the basolateral membranes (BLM) is mediated primarily by membrane potential-dependent organic cation transporters such as OCT1 (1) and OCT2 (2). OCT3 (3) may contribute in part to the cellular uptake of organic cations. Exit of cellular organic cations across brush-border membranes (BBM) is mediated principally by unidentified H^+/organic cation antiporter (4). P-glycoprotein (5) is involved in tubular secretion of hydrophobic drugs such as digoxin, anticancer agents, and some immunosuppressants (cyclosporine and tacrolimus).

membrane potential (inside negative) enhanced TEA uptake by isolated renal basolateral membrane vesicles, but not by brush-border membrane vesicles [70]. In rabbit renal basolateral membranes, both transmembrane potential-dependent uptake and organic cation exchange were found, but these two different modes of uptake appeared to be mediated by a single transport system rather than by two distinct systems [74, 75]. Smith, Pritchard, and Miller measured basolateral membrane potential and TEA uptake using flounder and killifish renal tubules, and found that depolarization of the basolateral membranes created by high K^+ or Ba^{2+} suppressed TEA uptake, and inversely that hyperpolarization stimulated TEA uptake [68]. Furthermore, the addition of TEA to the medium reversibly depolarized the basolateral membranes. These findings support the electrogenic facilitated transport of organic cations in renal basolateral membranes (Fig. 2).

Studies using isolated rat hepatocytes have suggested the existence of two different transport systems for organic cations: a type 1 system mediating uptake of small hydrophilic organic cations such as TEA, cimetidine, procainamide and guanidine, and a type 2 system mediating uptake of hydrophobic organic cations such as quinidine, quinine, d-tubocurarine, vecuronium, and cardiac glycosides [88, 89]. Most substrates of the type 2 system have potent inhibitory effects on the type 1 system. Although the uptake of type 2 substrates has not been directly demonstrated in renal basolateral membranes, such transport systems could exist, as renal secretion of type 2 cations such as quinine and quinidine has been observed [90]. Therefore, the multispecific organic cation transport systems in renal basolateral membranes may comprise more than a single transporter protein. Consistent with these functional studies, some organic cation transporters have been identified at the molecular level (discussed later in this article).

Functional properties of the organic cation transport system in renal brush-border membranes have been extensively investigated using isolated brush-border membrane vesicles [70–73, 76–83] and cultured renal epithelial cell lines [84–87]. A large body of data from these studies revealed that the transport system in these membranes is mediated by an electroneutral H^+/organic cation antiporter energized by transmembrane H^+ gradient, which can be sustained by the Na^+/H^+ exchanger and/or H^+-ATPase (Fig. 2). The H^+ and organic cation are tightly coupled as transient uphill uptake (overshoot phenomenon) of substrates such as TEA, and cimetidine was observed in the presence of an initial outward H^+ gradient (internal vesicle pH of 6.0 and external pH of 7.5), which was unaffected by the valinomycin- and K^+-induced transmembrane potential difference [70]. A stoichiometry of 1:1 was determined for H^+/organic cation exchange [70, 77]. The existence of an additional H^+/organic cation antiporter, which is more specific for guanidine, was demonstrated in the renal brush-border membranes of the rabbit [78]. This H^+/guanidine antiport activity was not inhibited by TEA and NMN. *Trans*-stimulation (cation/cation exchange activity) experiments and uptake studies showed that the H^+/organic cation antiporter mediates the translocation of a wide range of cationic compounds with diverse structures, such as endogenous cations including NMN and choline, and drugs including TEA, cimetidine, procainamide, neurotoxin 1-methyl-4-phenylpyridinium (MPP), and amino-β-lactam antibiotics [79–81, 83]. Therefore, the brush-border membrane H^+/organic cation antiporter appears to be a multispecific transporter for type 1 organic cations as well as the basolateral transporter. The brush-border H^+/organic cation antiporter shows substrate specificity that is similar, but not identical, to the basolateral membrane organic cation transporter [79]. Large and more hydrophobic compounds belonging to the type 2 cations are not translocated by the H^+/organic cation antiporter. Transport activity for such hydrophobic drugs and cations of the renal brush-border membranes appears to be relatively small, and MDR1/P-glycoprotein (Fig. 2, #5) and/or other transport mechanisms could be involved in the efflux of such compounds.

Several endogenous organic cations, such as monoamine neurotransmitters and choline, undergo renal tubular reabsorption as well as secretion, thereby maintaining their homeostasis [63, 91]. As bioactive mono-

amines such as dopamine and epinephrine have been suggested to participate in the reabsorption of Na^+ along lower nephron segments through an interaction with specific receptors, tubular uptake and efflux of monoamines may indirectly affect renal fluid balance. For some cationic compounds and xenobiotics, this reabsorption mechanism in the brush border membranes could be responsible for nephrotoxicity.

IDENTIFICATION OF ORGANIC CATION TRANSPORTERS

OCT family

In 1994, Gründemann et al identified the first member of the organic cation transporter family, designated as OCT1, from the rat kidney by expression cloning [92]. Rat (r)OCT1 is comprised of 556 amino acids with 12 putative transmembrane domains. Northern blot analysis showed that rOCT1 mRNA was expressed in the liver, kidney, and intestine. In the kidney, rOCT1 mRNA was detected in proximal tubules, glomeruli, and cortical collecting ducts, but not in distal tubules. By immunohistochemical analysis, rOCT1 was localized to the basolateral membranes of S1 and S2 segments of proximal renal tubules (Fig. 2, #1) and the small intestine and liver [9]. When expressed in oocytes, rOCT1 stimulated uptake of TEA, which was inhibited by diverse organic cations [92]. Electrophysiological experiments using rOCT1-expressing oocytes under voltage-clamped conditions demonstrated that positive inward currents were induced when TEA, NMN, choline, dopamine, or MPP were added to the bath medium, indicating that rOCT1-mediated cation uptake is electrogenic [93].

Human (h) OCT1 is comprised of 554 amino acids and shows 78% identity with rOCT1. Its mRNA transcript was detected exclusively in the liver [94]. There are distinct species differences in tissue distribution and histochemical localization of OCT1. After expression in oocytes, hOCT1 mediated the uptake of type 1 organic cations such as NMN, TEA, and MPP, suggesting that hOCT1 may primarily participate in hepatic excretion of organic cations in humans [95]. hOCT1-mediated MPP uptake was saturable with a Km value of 14.6 μmol/L and was sensitive to transmembrane potential. The type 2 hydrophobic cations such as vecuronium and decynium-22 as well as the type 1 hydrophilic cations such as TEA and NMN inhibited MPP uptake. hOCT1 has lower binding affinity for several cations such as decynium-22, tetrapentylammonium, quinine, and NMN than rOCT1, indicating species differences in the substrate specificity [95]. The human genes of hOCT1 and hOCT2 (also named SLC22A1 and SLC22A2) have been localized in close proximity on chromosome 6q26 [96].

Since OCT1 was cloned, other gene products with significant homology to OCT1 have been identified (Table 1). Using hybridization techniques, we isolated a cDNA encoding OCT2 from rat kidney [97]. rOCT2 is comprised of 593 amino acids with 12 proposed putative transmembrane domains showing a 67% identity to rOCT1. On Northern hybridization and RT-PCR analysis, the rOCT2 mRNA transcript was detected predominantly in the kidney, at higher levels in the medulla than the cortex, but not in the liver, lung, or intestine. When rOCT2 was expressed in oocytes, uptake of TEA was suppressed by the replacement of Na^+ with K^+, suggesting that the uptake was membrane potential-dependent [97]. Acidification of extracellular medium resulted in a decreased uptake of TEA, whereas the efflux of TEA out of rOCT1- and rOCT2-expressing oocytes was not stimulated by the inward H^+ gradient [98]. To compare the functional characteristics of rOCT1 and rOCT2, we established stable transfectants using MDCK cells [99]. TEA uptake by both rOCT1 and rOCT2 transfectants grown on microporous membrane filters was markedly enhanced when TEA was added to the basolateral bath medium, but not to the apical medium. TEA uptake by both transfectants was decreased by acidifying the medium pH, suggesting that rOCT1- and rOCT2-mediated TEA transport were pH sensitive. Efflux of TEA out of the transfectants was unaffected or moderately inhibited by acidification of the medium. Structurally diverse organic cations, including the type 1 cations such as MPP, cimetidine, NMN, nicotine, and procainamide, and type 2 cations, such as quinine and quinidine, inhibited TEA uptake in the transfectants [99]. Inhibition experiments suggested that rOCT1 and rOCT2 had similar inhibitor binding affinities for many compounds, but showed moderate differences in inhibitor sensitivity for several compounds such as MPP, procainamide, dopamine, and testosterone by a factor of 2 to 3 (unpublished data) [98, 99]. rOCT2 and hOCT2, which share 80% amino acid identity, have been shown to accept monoamine neurotransmitters such as dopamine, norepinephrine, epinephrine, 5-hydroxytryptamine, and amantadine as substrates [100, 101]. These findings raise the possibility that OCT2 plays a physiological role in renal handling of some bioactive monoamines and implies that the transporter is indirectly involved in the physiological function of these monoamines such as renal tubular reabsorption of Na^+.

Recently, we reported that slices and isolated basolateral membrane vesicles of male rat kidney showed a higher transport activity for TEA than those of female rat kidney [102]. The expression levels of rOCT2 mRNA and the protein in the kidney of males were much higher than those in females. There was no gender difference in mRNA expression levels of rOCT1. These findings suggested that rOCT2 is responsible for the gender differences in renal basolateral membrane organic cation transport activity (Fig. 2, #2) [102].

A cDNA encoding an additional member of the OCT gene family, designated as OCT3, was isolated from the rat placenta [103]. rOCT3 is comprised of 551 amino acids with 12 putative transmembrane domains and shows 48% identity to rOCT1. Northern blot analysis indicated that rOCT3 mRNA was detected most abundantly in the placenta and moderately in the intestine, heart, and brain. Expression of rOCT3 mRNA was comparatively low in the kidney and lung, and it was not detected in the liver. When expressed in HeLa cells and *Xenopus* oocytes, rOCT3 induced uptake of TEA and guanidine, which could be inhibited by MPP [103]. Under voltage-clamped conditions, rOCT3-mediated TEA uptake evoked a potential-dependent inward current. The current induced by the TEA uptake was markedly influenced by extracellular pH. However, such pH dependence of TEA uptake by rOCT3-expressing oocytes could not be confirmed under voltage clamp conditions. Therefore, rOCT3 appears to be a potential-sensitive and pH gradient-independent organic cation transporter (Fig. 2, #3). Although the distribution and localization of rOCT3 in the kidney have not yet been determined, it may also participate in the renal handling of a variety of organic cations.

By their homology to OCT transporters, two additional members of the OCT gene family, named hOCTN1 (SLC22A4) [104] and hOCTN2 (SLC22A5) [105, 106], have been identified (Table 1). A cDNA encoding hOCTN1 was cloned from human fetal liver and encodes 551 amino acid residue protein with 11 putative transmembrane domains and one nucleotide binding site motif [104]. hOCTN1 mRNA was found to be abundant in the kidney, trachea, bone marrow, fetal liver and several human cancer cell lines, but not in adult liver. When expressed in HEK293 cells, hOCTN1 mediated saturable and pH-dependent uptake of TEA with higher activity at neutral and alkaline than at acidic pH [107]. In addition, the efflux of TEA out of the cells was pH-dependent, with an accelerated rate at acidic external medium pH. TEA uptake was not influenced by membrane potential, and hOCTN1-mediated TEA uptake was inhibited by other organic cations such as cimetidine, procainamide, quinidine, quinine, and verapamil. When expressed in oocytes, hOCTN1 stimulated uptake of quinidine, verapamil, and zwitterionic L-carnitine [107]. The functional role of OCTN1 in the renal secretion of organic cations remains unknown.

hOCTN2 was identified as a homologue of hOCTN1 from human kidney. hOCTN2 cDNA encodes a 557-amino acid residue protein with 76% similarity to hOCTN1 [105, 106]. hOCTN2 is strongly expressed in the kidney, trachea, spleen, bone marrow, skeletal muscle, heart, and placenta in adult humans. When expressed in HEK293 cells, hOCTN2 mediated the uptake of L-carnitine in a Na^+-dependent manner with a Km value of 4.3 μmol/L, whereas it mediated some minor uptake of TEA and guanidine [106]. The physiological function of hOCTN2 is suggested to be a high-affinity Na^+-carnitine cotransporter. Nezu et al [108], Tang et al [109], and Wang et al [110] reported that primary systemic carnitine deficiency, which is an autosomal recessive disease characterized by low serum and intracellular concentrations of carnitine, is caused by mutations in the *hOCTN2* gene.

Interestingly, Wu et al reported that rOCTN2 is a Na^+-independent organic cation transporter as well as a Na^+-dependent carnitine transporter, which is expressed in the heart, kidney, placenta, and brain [111]. In rat kidney, rOCTN2 mRNA is predominantly expressed in the cortex, while there is very little expression in the medulla. In the cortical region, rOCTN2 mRNA was found in the proximal and distal tubules. There have been two mutations reported that result in amino acid substitution in OCTN2, P478L (hOCTN2) and L352R (mouse OCTN2) [111]. These mutations in hOCTN2 cause complete loss of carnitine transport function. In contrast, only the M352R mutant appeared to be associated with complete loss of organic cation transport function, whereas the P478L mutant had higher organic cation transport activity than the wild-type transporter. These studies suggested that the binding sites for carnitine and organic cations in OCTN2 exhibit significant overlap but are not identical. Therefore, there may be clinical implications for pharmacotherapy in individual patients with primary carnitine deficiency if the mutations in OCTN2 also affect organic cation transport activity.

MDR1/P-glycoprotein

MDR1/P-glycoprotein, a member of the ABC multidrug transporter superfamily, mediates active extrusion of drugs with diverse structures, such as vinca alkaloids, anthracyclines, steroids, cyclosporines, tacrolimus, and miscellaneous hydrophobic organic cations from the cell (Table 3) [112]. In the kidney, P-glycoprotein is expressed in the brush-border membranes of proximal tubules, where it pumps various hydrophobic xenobiotics into the lumen (Fig. 2, #5) [11]. The observation that digoxin, a cardiac glycoside, appears to be actively secreted by the renal proximal tubules via P-glycoprotein is of particular clinical importance for transporter-mediated drug interactions [113, 114].

FUNCTION AND STRUCTURE OF PEPTIDE TRANSPORTERS

Peptide transporters expressed in the brush border membranes of intestinal and renal epithelial cells are involved in the efficient absorption of oligopeptides, thereby contributing toward maintenance of protein nutrition [115]. The peptide transporters mediate an electrogenic H^+-coupled cotransport of dipeptides and tripeptides,

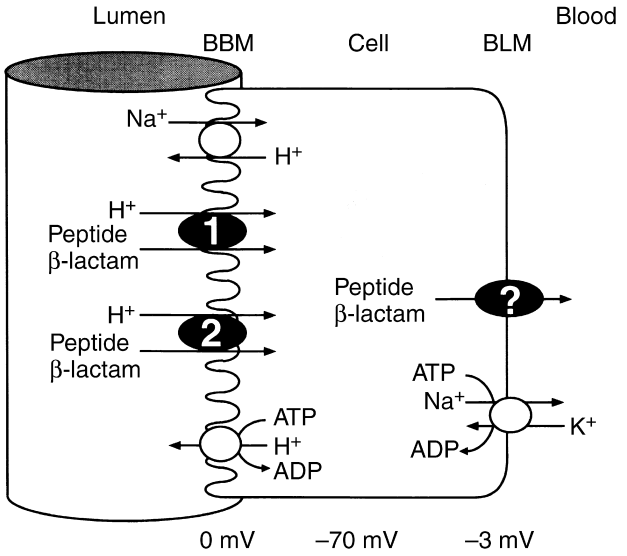

Fig. 3. Mechanisms of oligopeptide transport in renal tubular cells. Filtered oligopeptides and several β-lactam antibiotics are reabsorbed across brush-border membranes (BBM) by H^+-coupled oligopeptide cotransporters, low-affinity type PEPT1 (1) and high-affinity type PEPT2 (2). Cellular oligopeptides, which escape hydrolytic degradation by enzymes, and β-lactam antibiotics are translocated across basolateral membranes (BLM) by an unidentified peptide transporter.

which is driven by an inward H^+ gradient and a negative transmembrane potential difference [116]. Peptide transporters play pharmacological roles in some medications as they mediate transport of peptide-like drugs such as β-lactam antibiotics [83, 117, 118], angiotensin-converting enzyme (ACE) inhibitors [119] and the dipeptide-like anticancer drug bestatin [120, 121]. Studies using isolated membrane vesicles have shown that the uptake of glycylsarcosine in renal brush-border membranes is mediated by at least two distinct peptide transport systems: the high-affinity/low-capacity and the low-affinity/high-capacity systems (Fig. 3) [122]. The initial uptake rate of glycylsarcosine was *trans*-stimulated in brush-border membrane vesicles preloaded with cephalosporins with an α-amino group in molecules such as cephalexin, cephradine, and cyclacillin, suggesting that these antibiotics share the same peptide transporter [122].

Molecular cloning studies have identified that two homologous peptide transporters, designated as PEPT1 and PEPT2, are involved in mammalian epithelial transport of oligopeptides (Table 4). Fei et al identified rabbit PEPT1 from the intestine using expression cloning with *Xenopus* oocytes [123]. Rabbit PEPT1 is comprised of 709 amino acids, with 12 putative transmembrane domains. Rat [124] and human [125] homologues of rabbit PEPT1 have been isolated by hybridization techniques. Subsequently, cDNA encoding PEPT2 have been isolated from rabbit [126], rat [127], and human [128] kidneys. PEPT1 and PEPT2 show approximately 50% amino acid identity. By immunohistochemical studies, rPEPT1 was localized to the brush-border membranes in S1 segments of proximal tubules (Fig. 3, #1) [129] and brush border membranes along the digestive tract [130]. In contrast, rPEPT2 protein was detected primarily in brush border membranes of the S3 segments of proximal tubules (Fig. 3, #2) [129]. Therefore, rPEPT1 is expressed in early regions of the proximal tubules (pars convoluta), whereas rPEPT2 is specific for the later regions of proximal tubules (pars recta).

We compared the recognition of β-lactam antibiotics by LLC-PK_1 cells transfected with rPEPT1 or rPEPT2 cDNA [131, 132]. Cyclacillin (aminopenicillin) and ceftibuten (anionic cephalosporin without an α-amino group) showed potent inhibitory effects on glycylsarcosine uptake in the rPEPT1-expressing cells [131]. Other β-lactam antibiotics, such as cephalexin, cefadroxil, and cephradine (aminocephalosporins), showed a low inhibitory effect on rPEPT1-mediated glycylsarcosine uptake [132]. Except for ceftibuten, these antibiotics had a much more potent inhibitory effect on glycylsarcosine uptake via rPEPT2 than via rPEPT1. rPEPT2 was concluded to have a much higher affinity for β-lactam antibiotics with an α-amino group than rPEPT1, and substituents at the NH_2-moiety of these drugs are responsible for recognition by both peptide transporters. In addition, we found that the α-amino group of β-lactam antibiotics interacts with the histidine residues of rPEPT1 and rPEPT2 and is involved in the mechanism of substrate recognition by these peptide transporters [133]. Site-directed point mutation analysis has suggested that both histidine residues at positions 57 and 121 of rPEPT1, which are conserved in the rat, rabbit, and human PEPT1, participate in the recognition of β-lactam antibiotics by the transporter [134].

Quinapril, an ACE inhibitor, was found to inhibit uptake of glycylsarcosine by rabbit renal brush-border membrane vesicles [135]. The inhibition constant (Ki value) of approximately 1 mmol/L was several-fold higher than the Km value for glycylsarcosine, and the interaction appeared to be noncompetitive. On the other hand, enalapril, another ACE inhibitor, was shown to inhibit uptake of glycylsarcosine in a competitive manner with a Ki value of 6 mmol/L [136]. Although the ACE inhibitors can interact with renal high-affinity peptide transporter, PEPT2, it is still not known whether renal reabsorption of ACE inhibitors across the brush border membranes is mediated by the peptide transporters.

Valacyclovir, a valyl ester prodrug of the antiviral agent acyclovir, does not contain a peptide bond in its structure, but it is transported by PEPT1 [137]. Valacyclovir competitively inhibited glycylsarcosine uptake in rPEPT1- or rPEPT2-expressing LLC-PK_1 cells [138]. rPEPT2 had higher affinity for this agent (Ki value of 0.22 mmol/L) than rPEPT1. Among several L-amino acid

Table 4. Peptide transporter family

Transporter	Species	Accession	Chromosome	Tissue distribution	Substrates[a]
PEPT1	Human	U13173	13q24-q3	Small intestine>kidney (rat)	di-, tripeptides, β-lactam antibiotics, bestatin, ACE inhibitors, valacyclovir
	Rabbit	U06467			
	Rat	D50306			
PEPT2	Human	S78203	3q13.3-q21	Kidney≫brain, lung, spleen (rat)	di-, tripeptides, β-lactam antibiotics, bestatin, ACE inhibitors
	Rabbit	U32507			
	Rat	D63149			

[a] The substrate specificities were examined for each rat clone

methyl esters examined, L-valine methyl ester, as well as valacyclovir, appeared to have high affinity for both rPEPT1 and rPEPT2 [138]. From the viewpoint of drug delivery, L-valyl esterification of poorly absorbed drugs has been postulated to be a useful strategy for improving their bioavailability and therapeutic efficacy.

CLINICAL IMPLICATIONS OF TRANSPORTER–MEDIATED DRUG INTERACTIONS

Organic anion and cation transporters in the kidney are involved in the pharmacological responses to certain drugs such as diuretics, and in drug–drug interactions, and clinically, this is of therapeutic or toxic importance [139]. It is well known that probenecid inhibits renal secretion of other diverse anionic drugs through the organic anion transport system(s), resulting in decreased drug excretion. Renal excretion of ciprofloxacin, a fluoroquinolone antibacterial drug, was decreased by coadministration of probenecid in humans [140]. It has been suggested that this type of drug interaction could be of clinical relevance for the combined use of ciprofloxacin and drugs being transported by the organic anion transporter in the kidney. The interaction between methotrexate, which has been widely used for the treatment of acute leukemia, psoriasis, and rheumatoid arthritis, and NSAIDs has been reported with severe adverse effects after chemotherapeutic use [141]. It is assumed that the basolateral membrane OAT1 and brush-border membrane OAT-K1 and/or OAT-K2 are the organic anion transporters involved in the methotrexate–NSAIDs interaction in the kidney [51].

Cimetidine and trimethoprim appeared to be potent inhibitors of the renal tubular secretion of a number of cationic drugs, particularly procainamide and its active metabolite N-acetylprocainamide, causing significant clinical toxicity [142]. In therapeutic doses, other histamine H_2 receptor antagonists may have minimal effects on renal procainamide elimination. Using the isolated perfused rat kidney model, H_2 antagonists such as ranitidine and famotidine were suggested to have inhibitory effects on the renal secretion of triamterene [142]. These findings suggested that renal tubular secretion of triamterene is mediated by the organic cation transporter, whereas the secretion of p-hydroxytriamterene sulfate, a metabolite of triamterene, is via the organic anion transporter.

The clinical use of digoxin has been complicated by drug interactions leading to severe drug toxicity. Using LLC-PK_1 cells transfected with human MDR1 cDNA, P-glycoprotein appeared to induce transepithelial transport of digoxin, corresponding to tubular secretion, and be responsible for the digoxin–quinidine interaction [114]. The interaction of digoxin and clarithromycin, a potent macrolide antibiotic, has been observed in two patients in whom plasma concentrations of digoxin were unexpectedly elevated. In the MDR1/P-glycoprotein-expressing transfectant, clarithromycin inhibited transcellular transport of digoxin and concomitantly increased cellular accumulation of digoxin [143]. These findings suggested that clarithromycin inhibits the P-glycoprotein-mediated tubular secretion of digoxin, thereby leading to an increase in the plasma digoxin concentration.

Besides the direct interaction of drugs with drug transporters described previously in this article, indirect modulation of transporter proteins could be involved in kidney–specific drug interactions. In the case of organic anion transporter OAT1, protein kinase C activation results in a marked decrease in its transport function [28]. Therefore, agents that modulate protein kinase C activity could affect the renal disposition of anionic drugs via inhibitory regulation of OAT1. For additional modification of organic anion transporter, a selective adenosine A_1 receptor antagonist, KW-3902, was reported to have an inhibitory effect on PAH transport in OK cells [144]. Staurosporin, a protein kinase inhibitor, did not restore the KW-3902–induced decrease in PAH transport activity, suggesting that protein kinase C may not be involved in its inhibitory effect. These findings are examples of indirect drug interaction based on modulation of renal drug transporters rather than competitive and/or noncompetitive interactions. In addition, compounds including drugs, xenobiotics, and endogenous factors, which either affect or regulate the level of expression of drug transporters, could influence renal handling of therapeutic drugs being secreted or reabsorbed in the kidney.

PERSPECTIVES

Current information regarding molecular and cellular aspects of drug transporters in the kidney has facilitated studies on mechanisms of renal disposition as well as transporter-mediated drug interactions. A large number of drug transporters such as OAT and OCT families (Table 1), oatp family (Table 2), ABC transporter superfamily (Table 3), and PEPT family (Table 4), have been identified, and the number of members of these families will likely be increased. Clarification of the physiological as well as the pharmacological role of each cloned transporter in renal handling of drugs in vivo is of potential importance. Species differences in substrate selectivity, tissue distribution, and level of expression of the drug transporters should be explored to aid in the prediction of the in vivo kinetic profile of drugs from in vitro data. Construction of the in vitro system using human drug transporters, which could be reconstituted with human cDNA clones, may serve as a useful model for the prediction of transporter-mediated drug interactions. As described in primary systemic carnitine deficiency, mutations of human OCTN2 belonging to the OCT gene family were discovered and proved to be the responsible gene for this genetic disease [108–110]. There could be mutations in other members of this gene family. Mutations in drug transporters causing transport deficiency or complete loss of transporter protein would be of clinical and pharmacological importance. Inversely, the genetic mutation involved in deficient transport function may provide valuable information about the structure–function relationship of drug transporters. Studies on polymorphisms of human drug transporters have been recently initiated, and in the future, the information obtained could be used for establishing appropriate medication for individual patients.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin-converting enzyme; ABC, ATP-binding cassette; α-KG, α-ketoglutarate; BSP, sulfobromophthalein; cMOAT, canalicular multispecific organic anion transporter; EHBR, Eisai hyperbilirubinemic; MDR, multidrug resistance; MDCK, Madin-Darby canine kidney; MPP, 1-methyl-4-phenylpyridinium; MRP, multidrug resistance-associated protein; NLT, novel liver-specific transport protein; NMN, N^1-methylnicotinamide; NSAID, nonsteroidal anti-inflammatory drug; OAT, organic anion transporter; oatp, organic anion-transporting polypeptide; OCT, organic cation transporter; OK, opossum kidney; PAH, para-aminohippurate; PEPT, peptide transporter; RT-PCR, reverse transcription-polymerase chain reaction; TEA, tetraethylammonium.

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