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Molecular Aspects of Organic Ion Transporters in the Kidney

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A function of the kidney is elimination of a variety of xenobiotics ingested and wasted endogenous compounds from the body. Organic anion and cation transport systems play important roles to protect the body from harmful substances. The renal proximal tubule is the primary site of carrier-mediated transport from blood into urine. During the last decade, molecular cloning has identified several families of multispecific organic anion and cation transporters, such as organic anion transporter (OAT), organic cation transporter (OCT), and organic anion-transporting polypeptide (oatp). Additional findings also suggested ATP-dependent organic ion transporters such as MDR1/P-glycoprotein and the multidrug resistance-associated protein (MRP) as efflux pump. The substrate specificity of these transporters is multispecific. These transporters also play an important role as drug transporters. Studies on their functional properties and localization provide information in renal handling of drugs. This review summarizes the latest knowledge on molecular properties and pharmacological significance of renal organic ion transporters.

Key Words: Organic anion, Organic cation, Multispecific organic anion transporter, Organic cation transporter, Organic anion transporting polypeptide, Multidrug resistance protein, P-glycoprotein, Tubular secretion, Xenobiotics

INTRODUCTION

The kidney and liver are highly developed organs for excretion of amphiphilic organic anions and cations with diverse chemical structures. Many of these anions and cations are endogenous and exogenous compounds, including a number of clinically used drugs, pesticides, and herbicides. These compounds are chemically heterogeneous substances possessing a carbon backbone and a net negative or positive charge at physiological pH. A great variety of endogenous and exogenous substances that are harmful to the body can be classified into organic anions and organic cations, and their elimination is therefore essential for the maintenance of homeostasis. Excretory organs such as the kidney, liver and intestine defend the body against potentially harmful effects of these compounds by biotransformation into less active metabolites and the excretory transport process. Particularly in the kidney, drugs and environmental toxicants are eventually excreted into the urine, either in the unchanged form or as biotransformation products. This renal excretion is closely related to physiological events occurring in nephrons, i.e., filtration, secretion, and reabsorption. More than 70 years ago, it was already observed that phenolsulfonphthalein, an anionic dye, was highly concentrated in the renal convoluted tubules, indicative of the tubular secretion process (Marshall & Vicker, 1923). Transport systems responsible for renal tubular secretion of endogenous or exogenous compounds have been divided into either the organic anion system or the cation transport system based on their preferential substrate selectivity (Roch-Ramel et al, 1992; Ullrich, 1994). Renal secretory transport is performed 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 apical membrane to mediate elimination of cellular substrates into the urine (Pritchard & Miller, 1993; Pritchard & Miller, 1996). In addition to these organic

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anion transporters (OAT) and organic cation transporters (OCT), adenosine-triphosphate binding cassette (ABC) proteins, such as members of the multidrug resistance-associated protein (MRP) (Schaub et al, 1997; Kool et al, 1999) and P-glycoprotein (Fojo et al, 1987; Thiebaut et al, 1987) families have been suggested as the drug export pumps in renal tubules. During the last decade, renal transporters of organic anions and cations that share structural properties have been cloned. This review will focus on the present knowledge of molecular aspects of renal organic ion transporters.

Functional properties of organic anion transport system

Various organic anions are secreted from the renal proximal tubules. In the renal proximal tubules, the active transepithelial secretion of organic anions into the urine is mediated by two types of transporter proteins existing in the basolateral and luminal membranes. The first step is the uptake. Tubular secretion of organic anions occurs in two transmembrane transport processes (Fig. 1). Considering the electrical potential inside a cell of the proximal tubule, the basolateral uptake of organic anions is an uphill transport process. In 1987, Shimada and coworkers reported that uptake of para-aminohippuric acid (PAH), a prototype substrate of renal organic anion transport system, was markedly enhanced in the presence of an outward α-ketoglutarate gradient (Shimada et al, 1987). PAH is taken up by proximal tubule cells in exchange for intracellular dicarboxylates, which are then returned to the cells via the Na^+-dicarboxylate cotransporter. These findings indicate that the PAH transporter is a tertiary active transporter. Na^+, K^+ ATPase (a primary active transporter) generates the sodium ion gradient between the plasma membranes (Pritchard, 1995), and the electrochemical gradient of the sodium ions derives the transport of dicarboxylates via the Na^+-dicarboxylate cotransporter (a secondary active transporter) (Pritchard, 1988). Using the gradient of dicarboxylates (inside > outside), the PAH transporter takes up

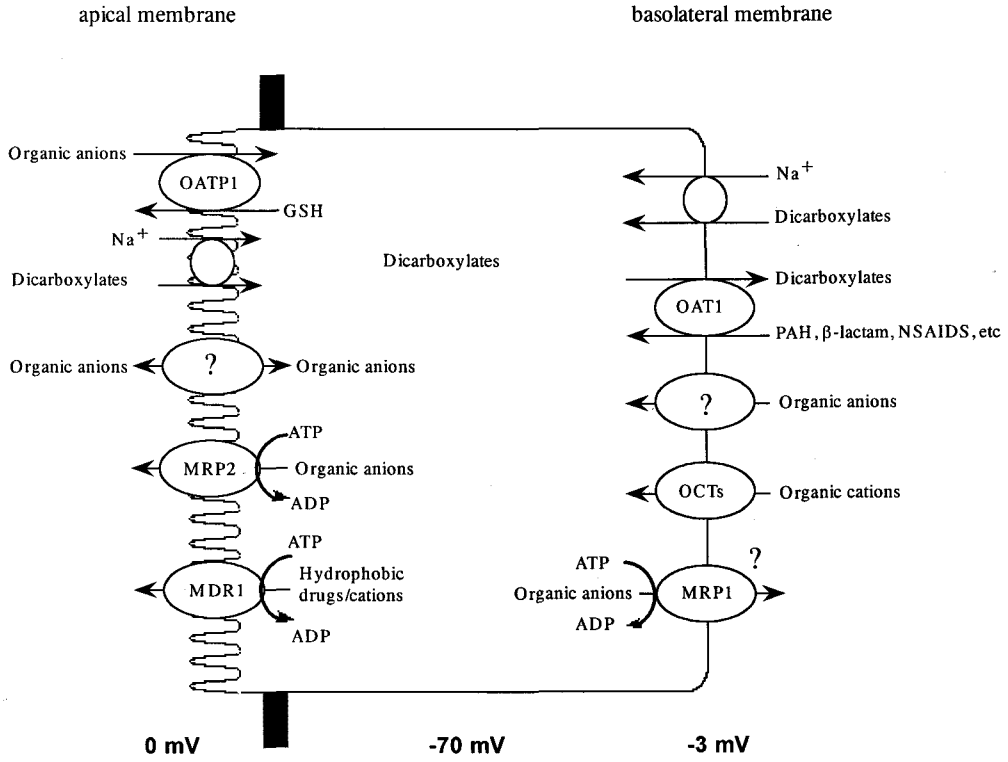

Fig. 1. Mechanisms of organic ions transport in renal tubular cells. Cellular uptake of organic anions and cations across basolateral membrane is mediated by organic anion and cation transporters. MRP2 and MDR1 may contribute to tubular secretion of anionic or cationic conjugates of hydrophobic compounds.

organic anions in exchange for the dicarboxylates (a tertiary active transporter). In addition to this classical PAH transporter, an additional uptake system has been characterized using bulky organic anion fluorescein-methotrexate as a substrate (Masereeuw et al, 1996). Uptake of fluorescein-methotrexate is independent of Na^+ and is not inhibited by PAH or glutarate. The molecular identity of this transporter has not yet been established. Finally, ABC also has been extensively characterized as an export system for organic anions (Schaub et al, 1997).

Molecular structure of organic anion transporter

We and Sweet et al. independently isolated the PAH transporter from rat kidney (OAT1/ROAT1) in 1997 using the expression cloning technique (Sekine et al, 1997; Sweet et al, 1997). OAT1 and ROAT1 are identical and will be collectively referred to as OAT1. OAT1 is a 551-amino-acid protein with 12 putative membrane-spanning domains (Fig. 2). No biochemical investigation on the transmembrane topology has been reported yet. Other OATs have 535-568 amino acid residues. The molecular structure of OAT1 has no similarity to those of other members of polyspecific organic anion transporter families, e.g., the organic anion transporting polypeptide (oatp) or the multidrug resistance-associated protein (Mrp) families. On the other hand, the amino acid sequence alignment shows a low, but distinct, degree of homology with other isoforms of OAT, OCT and OCTN/carnitine transporters (Table 1).

All OATs have several N-glycosylation sites between the first and second transmembrane domains. Inhibition of glycosylation by tunicamycin in mouse

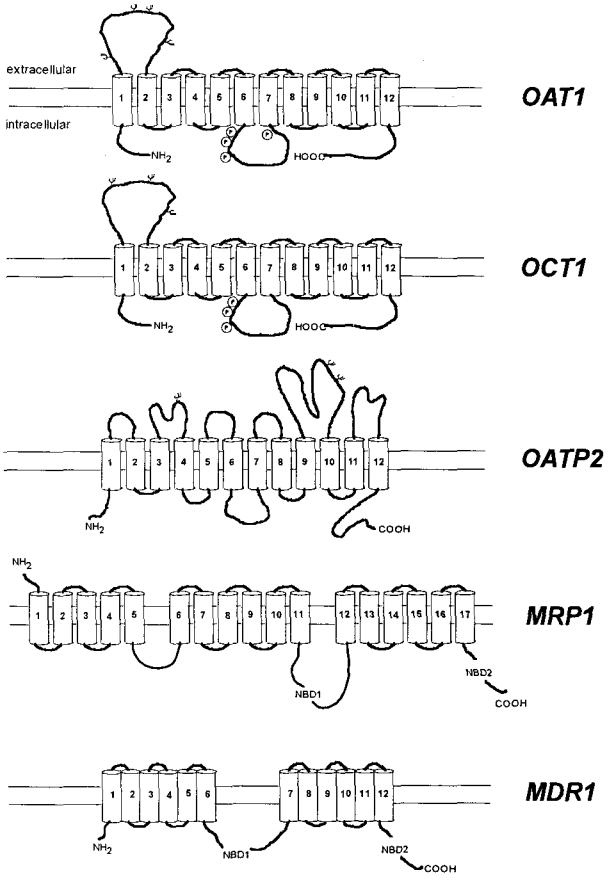

Fig. 2. Two-dimensional membrane topology models of represenative organic ion transporters. OAT, OCT, OATP and MDR1 show both N-terminal and C-terminal in intracellular site. In MRP, N-terminal and C-terminal is present extracellular and intracellular sites, respectively. MRP and MDR have two nucleotide binding domains (NBD) in intracellular site.

Table 1. Percent amino acid identity between fully sequenced organic ion transporters

	rOAT2	rOAT3	hOAT4	rOCT1	rOCT2	rOCT3	hOCTN1	hOCTN2	rCT1
rOAT1	42	49	43	38	36	36	34	36	36
rOAT2		39	38	36	36	37	36	37	37
rOAT3			43	36	35	35	36	36	37
hOAT4				35	32	36	35	36	37
rOCT1					64	48	38	39	38
rOCT2						48	37	39	38
rOCT3							36	37	36
hOCTN1								76	73
hOCTN2									85

OAT1-expressing COS-7 cells resulted in an increase of intracellular accumulation of newly synthesized transporters, suggesting that glycosylation is required for insertion of OAT1 into the plasma membrane (Kuze et al, 1999).

In all members of the OAT family, several protein kinase C (PKC) phosphorylation sites are predicted in the large loop between the sixth and seventh transmembrane domains (Fig. 2). The transport function of OAT1 is regulated by PKC. The PAH transport by rOAT1 (Uwai et al, 1998) and hOAT1 (Lu et al, 1999) expressed in *Xenopus laevis* oocytes and in HeLa cells, respectively, was inhibited after treatment of cells with phorbol esters. This phorbol-ester-induced inhibition of PAH transport was prevented by staurosporin, suggesting an inhibitory role of protein kinase C in OAT1. In addition, the putative phosphorylation sites for protein kinase A, casein knase II, and tyrosine kinase have also been reported. Whether any of these sites is used for the regulation of OATs is yet unclarified.

Functional characterization and tissue distribution of OATs

OAT1: OAT1 has been characterized functionally using *Xenopus oocytes* and the cell expression system (Table 2). The cloned OAT1 has been expressed in *X. laevis* oocytes (Sekine et al, 1997; Sweet et al, 1997; Wolff et al, 1997; Burckhardt et al, 1998; Uwai et al, 1998; Hosoyamada et al, 1999; Race et al, 1999), COS-7 cells (Kuze et al, 1999), or HeLa cells (Lu et al, 1999). The uptake ratio of radiolabeled PAH in cells expressing these carriers was increased compared with that of water-injected oocytes or mock-transfected cells, respectively. The reported Km values range between 5 and 70 μM [5 and 9 μM for hOAT1 (Hosoyamada et al, 1999; Lu et al, 1999); 37 μM for mOAT1 (Kuze et al, 1999); 14 and 70 μM for rOAT1 (Sekine et al, 1997; Sweet et al, 1997) and 21 μM for flounder OAT1 (Wolff et al, 1997)]. Uptake of PAH by rOAT1, hOAT1, and fOAT1 was trans-stimulated by intracellulary accumulated α-ketoglutarate or glutarate. Similarly, efflux of radiolabeled glutarate from OAT1-expressing oocytes was trans-stimulated by PAH in the incubation medium (Apiwattanakul et al, 1999). It was proved that OAT1 acts as a PAH/dicarboxylate antiporter. In addition to PAH, rOAT1 mediated the transport of various organic anions, including endogenous organic anions, such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), prostaglandin E2 (PGE2), dicarboxylic acid and urate, and exogenous ones, including various anionic drugs such as β-lactams, nonsteroidal antiinflammatory drugs (NSAIDs), antiviral drugs, and methotrexate (Sekine et al, 1997; Uwai et al, 1998; Apiwattanakul et al, 1999; Jariyawat et al, 1999; Sekine et al, 1999; Wada et al, 2000; Sekine et al, 2000). The affinities of these compounds for OAT1 were very similar to those reported for the basolateral PAH transporter. In addition, the hydrophobicity and the strength of the anionic charge of substrates appear to be closely related to their affinity for OAT1 (Ullrich, 1997). OAT1 also transports ochratoxin A (Tsuda et al, 1999), which is well known as the etiologic agent responsible for Balkan nephropathy. When OAT1-expressing cells and mock-transfected cells were exposed to ochratoxin A, decreased cell viability was observed in OAT1-expressing cells, which were rescued by inhibition of the OAT1-mediated uptake of ochratoxin A (Tsuda et al, 1999). This result suggests that OAT1 is associated with proximal tubular damage caused by anionic compounds (Endou, 1998).

Recently, an electrophysiological approach was used to demonstrate translocation of organic anions by fOAT1-expressing Xenopus oocytes (Burckhardt et al, 2000). PAH, bumetanide, etacrynic acid, and tienilic acid evoked an inward current. In contrast to these compounds, probenecid and furosemide did not generate an inward current. Whether rat or human OAT1 does transport furosemide is unknown at present.

The tissue distribution of cloned rat, mouse, and human OAT1 is investigated by northern blot analysis (Table 2) (Sekine et al, 1997; Sweet et al, 1997; Uwai et al, 1998; Hosoyamada et al, 1999; Kuze et al, 1999; Lu et al, 1999; Race et al, 1999). rOAT1, mOAT1, and hOAT1 mRNAs are expressed predominantly in the kidney and very weakly in the brain. In the rat kidney, rOAT1 is localized in the middle portion of proximal tubules (S2 segment) as determined by in situ hybridization (Sekine et al, 1997). Immunohistochemical localization also demonstrated that the rat (Tojo et al, 1999; Nakajima et al, 2000) and human OAT1 (Hosoyamada et al, 1999) proteins are restricted to the S2 segment of proximal tubular cells. Western blot analysis revealed that the rOAT1- encoded protein size was about 77 kDa.

OAT2: OAT2 has been cloned from the membrane

Table 2. Molecular characteristics of organic anion transporters in the kidney

Trnasporter (HGNC name)	Species	Accession	Chromosome	Tissue distribution	Substrate
OAT1# (SLC22A6)	Human	AB009697	11q11-11q13.1	kidney, brain	PAH, α-KG, cAMP
	Rat	AB004559		kidney, brain	various anionic drugs
	Mouse	U52842	19	kidney, brain	
	Winter flounder	Z97028		kidney	
OAT2# (SLC22A7)	Human	AF097518			α-KG, salicylate,
	Rat	L27651		kidney, liver	PAH, dicarboxylate
OAT3# (SLC22A8)	Human	AF097491	11q12-q13.3	kidney, liver, brain, eye	PAH, ochratoxin A,
	Rat	AB017466	19		estrone sulfate, cimetidine
OAT4*	Human	AB026198		kidney, placenta	estrone sulfate, DHEA-sulfate, ochratoxin A
oatp1# (SLC21A1)	Rat	L19031		kidney, liver	BSP, ouabain, leukotriene C4, etc
oatp2# (SLC21A5)	Rat	U88036		kideny, liver, retina	digoxin, ouabain, b-estradiol glucuronide
oatp3# (SLC21A7)	Rat	AF041105		kidney, retina, liver	taurocholate, thyroxine, 3,5,3'-triiodo-l-thyronine
OATP* (SLC21A3)	Human	U21943	12	brain	BSP, cholate, glycholic acid
OAT-K1# (SLC21A4)	Rat	D79981		kidney	MTX, folate
OAT-K2# (SLC21A4)	Rat	AB012662		kidney	MTX, folate, PGE2, taurocholate

HGNC: HUGO Gene Nomenclature Committee
and * was examined subustrate specificity for each rat and human clone, respectively.
PAH, p-aminohippurate; α-KG, α-ketoglutarate; DHEA, dehydroepiandrosterone; BSP, sulfobromophthalein; MTX, methotrexate

protein previously called the novel liver transporter (NLT), but whose transport substrates had not been identified (Simonson et al, 1994). Rat OAT2 had 42% amino acid sequence homology with rOAT1, and was shown to be a member of the OAT family (Table 1) (Sekine et al, 1998). rOAT2 mediated the sodium-independent transport of salicylate (Km=88 μM) and α-ketoglutarate (Km=18 μM). rOAT2 also mediated the transport of PGE2, methotrexate, acetylsalicylate, and PAH (Table 2). Mouse or human orthologs are not identified at present. rOAT2 is a 535-amino-acid protein with 12 putative membrane-spanning domains. rOAT2 mRNA is predominantly expressed in the liver, and weakly in the kidney.

OAT3: Rat OAT3, another member of the multispecific organic anion transporter family, was isolated from the rat brain by the RT-PCR cloning method based on the sequence conserved among rOAT1, rOAT2 and rat organic cation transporter 1 (rOCT1) (Kusuhara et al, 1999). rOAT3 shows 49, 39, and 36% identity with rOAT1, rOAT2, and rOCT1, respectively (Table 1). When expressed in

Xenopus oocytes, rOAT3 mediated the uptake of organic anions such as estrone sulfate (Km=2.3 μM), ochratoxin A (Km=0.74 μM), and PAH (Km=65 μM), and cimetidine (Table 2), known as a bisubstrate of OAT and OCT. OAT3 also interacted with anionic neurotransmitter metabolites, such as epinephrine, norepinephrine, and serotonin. The transport mode of OAT3 was sodium-independent, and no trans-stimulatory effect of estrone sulfate, PAH, or ochratoxin A was detected. When rOAT3 is expressed in cells, rOAT3-mediated estrone sulfate uptake is enhanced compared with that in the case of mock-transfected cells. This estrone sulfate uptake mediated by rOAT3 was regulated by protein kinase C (Takeda et al, 2000). In 1999, Race et al. reported hOAT3 (1999). They failed to detect any functional properties of hOAT3. rOAT3 mRNA was expressed in the liver, kidney and brain, and a weak expression was detected in the eye (Kusuhara et al, 1999). hOAT3 was detected mainly in the kidney and very weakly in the brain and smooth muscle. hOAT3 was distributed on the basolateral membrane of proximal tubule cells (Cha et al, 2001).

OAT4: Recently, we identified the fourth member of organic anion transporter expressed in the human placenta using EST (expressed sequence tag) data base searched for "query OAT1" (Cha et al, 2000). OAT4 is a 551-amino-acid protein with 12 putative membrane-spanning domains. hOAT4 showed 38 to 44% identity with those of other members of the OAT family (Table 1). When expressed in *Xenopus* oocytes, OAT4 mediated the high-affinity transport of estrone sulfate (Km=1.01 μM) and dehydroepiandrosterone sulfate (Km=0.63 μM) in a sodium-independent manner (Table 2). OAT4 also mediated the transport of ochratoxin A and PAH. OAT4-mediated transport of estrone sulfate was inhibited by several sulfate conjugates, such as *p*-nitrophenyl sulfate, α-naphthyl sulfate, β-estradiol sulfate, and 4-methylumbelliferyl sulfate. By contrast, glucuronide conjugate showed little or no inhibitory effect on the OAT4-mediated transport of estrone sulfate. Northern blot analysis revealed that OAT4 mRNA is abundantly expressed in the placenta as well as in the kidney. OAT4 is the first member of the multispecific organic anion transporter family that is expressed abundantly in the placenta. OAT4 might be responsible for the elimination and detoxification of harmful anionic substances from the fetus.

Organic anion transporting polypeptide (oatp) family

In 1994, oatp1 was isolated from the rat liver by expression cloning as a sodium-independent sulfobromophthalein (BSP) transporter Oatp1 encoded 670 amino acids with 10 putative transmembrane domains (Jacquemin et al, 1994). Oatp1 shows a markedly wide substrate selectivity and mediated the transport of conjugated and unconjugated bile salts, steroid hormones (Bossuyt et al, 1996) and various organic anions (Kontaxi et al, 1996; Meier et al, 1997; Li et al, 1998). Northern blot analysis revealed the expression of oatp1 mRNA in the rat liver, kidney, brain, lung, skeletal muscle, and proximal colon. Immunohistochemical study of the liver revealed the sinusoidal plasma membrane localization of oatp1 (Bergwerk et al, 1996). In the kidney, immunostaining of oatp1 was observed in renal brush-border membranes in the S3 segment of the proximal tubule (Bergwerk et al, 1996). Two additional cDNAs encoding other members of the oatp family, oatp2 (Noe et al, 1997) and oatp3 (Table 2) (Abe et al, 1998) were isolated from the rat brain and retina, respectively. Oocytes injected with cRNA encoding oatp2 and oatp3 showed uptake of taurocholate, thyrosine, and triiodo-thyronine in a saturable manner. In addition, the uptake of cardiac glycosides such as digoxin and ouabain was stimulated by oatp2. The oatp2 mRNA was found to be widely expressed in the central nervous system, neuronal cells as well as in the retina and liver. In contrast to oatp2 mRNA, oatp3 mRNA was expressed in the kidney and moderately in the retina. In 1996, a cDNA encoding a member of the oatp gene family, designated as OAT-K1, was isolated (Saito et al, 1996). Rat OAT-K1 consists of 669 amino acids and shows 72% identity with rat oatp1. When OAT-K1 cDNA was transfected stably to LLC-PK1 cells, OAT-K1 mediated the uptake of methotrexate (Km=1 μM) and folate in a sodium- independent manner, but not that of PAH, taurocholate, prostaglandin E2, or leukotriene C4. In OAT-K1-expressing cells, indomethacin and ketoprofen inhibited the transport of methotrexate in a competitive manner (Masuda et al, 1997). Northern hybridization analysis showed that OAT-K1 mRNA was expressed exclusively in the kidney. In LLC-PK1 cells transfected with OAT-K1, the transporter protein was localized in the basolateral membranes. In the kidney, OAT-K1 mRNA was found predominantly in superficial and juxtamedullary pro-

ximal straight tubules by the RT-PCR technique (Masuda et al, 1997). Another kidney-specific isoform of oatp was isolated from the rat kidney (Masuda et al, 1999). OAT-K2 consists of 498 amino acids and shows 91% identity with rat OAT-K1. OAT-K2 mediated the uptake of various organic anions such as taurocholate, methotrexate, folate, and prostaglandin E2. OAT-K2 mRNA was detected in proximal convoluted and straight tubules and cortical collecting ducts. In MDCK cells transfected with OAT-K2, the transporter protein was localized functionally to the apical membranes.

Multidrug resistance-associated protein (MRP) family

MRP1: MRP1 is identified from a human multidrug-resistant lung cancer cell line as a glutathione S-conjugate pump (Cole et al, 1992). MRPs belong to the large family of ATP-binding cassette proteins and function as ATP-dependent transporters of anionic conjugates, as well as drug conjugates and unmodified anticancer drugs such as leukotriene C4, S-(dinitrophenyl)-glutathione, estradiol-17 β-D-glucuronide, etoposide-glucuronide, and daunorubicin (Fig. 1, Fig. 2 and Table 3) (Evers et al, 1996; Keppler et al, 1999). Murine Mrp1 is localized to the basolateral membrane of various epithelial cells, and human MRP1 is sorted to the basolateral membrane when expressed in the kidney originated epithelial cell lines LLC-PK1 and MDCK II (Evers et al, 1996; Evers et al, 1997). Murine Mrp1 is expressed at the basolateral membrane of cells of Henle's loop and the cortical collecting duct.

MRP2/cMOAT: MRP2 was found to be defective in mutant rats, such as Eisai hyperbirubinemic (Buchler et al, 1996; Ito et al, 1997) and TR-rat (Table 3) (Paulusma et al, 1996). The absence of MRP2 in humans results in the Dubin-Johnson syn-

Table 3. Molecular characteristics of ABC transporter family

Trnasporter (HGNC name)	Species	Accession	Chromosome	Tissue distribution	Substrate
MRP1 (ABCC1)	Human	L05628	16p13.1	ubiquitous	anticancer agents, anionic conjugates
	Mouse	AF022908		ubiquitous	
MRP2 (ABCC2)	Human	U49248	10q24	kidney, liver, nerve, small intestine	bilirubin glucuronides
	Rat	AB017446		kidney, liver, small intestine	
MRP3 (ABCC3)	Human	AB010887	17q21.3	liver, small intestine	anionic conjugates
	Rat	AB010467		kidney, colon, lung, small intestine	
MRP4 (ABCC4)	Human	U83660	13q31-32	ubiquitous	
MRP5 (ABCC5)	Human	U83661	3q27	ubiquitous	
	Mouse	AB019003			
MRP6 (ABCC6)	Human	AF076622	16p13	kidney, liver	
	Rat	AB010466		kidney, liver, duodenum	
	Mouse	AB028737			
MDR1/P-glycoprotein (ABCB1)	Human	M14758	7q21	liver, small intestine, kidney, brain, adrenal gland	Hydrophobic (cationic) compounds
	Rat	L15079			anticancer agents,
	Mouse	M33581, J03398			digoxin, etc

The substrate specificities were examined for each human clone.

drome (Wada et al, 1998). MRP2 is the most extensively studied member of MRPs with 48% identity with MRP1. Mrp2 also transports glutathione S-conjugate leukotriene C4 and the glucuronide conjugate of bilirubin. Furthermore, studies of 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 (Leier et al, 2000; Van Aubel et al, 2000). Immunohistochemical analysis showed that MRP2 was localized to the brush-border-membranes of proximal tubules (segment S1, S2, and S3). MRP2 in the kidney has been suggested to contribute to cellular detoxification and secretion of endogenous and xenobiotic anionic compounds from blood into the urine (Schaub et al, 1997).

Other member of MRPs (MRP3, MRP4, MRP5, and MRP6)

From a search of GenBank database EST (expressed sequence tag), four additional human MRP-like genes were isolated and reported (Table 3). When human MRP3 was overexpressed in MDCK II cells, MRP3 extruded of S-(dinitrophenyl)-glutathione across the basolateral membrane compared with that when MRP3 was expressed in the parental cells (Kool et al, 1999). In a T-lymphoid cell line, overexpression of human MRP4 mRNA and the MRP4 protein was found to be correlated with enhanced ATP-dependent efflux of nucleoside monophosphate analogs (Schuetz et al, 1999). Substrate specificity of MRP5 has been investigated by expression of a conjugate of green-fluorescence protein in HEK-293 cells (McAleer et al, 1999). In a recent report, rat Mrp6 was observed to mediate the ATP-dependent transport of the anionic endothelin antagonist BQ-123 (Madon et al, 2000). Expression of MRP3 (Belinsky et al, 1998; Kiuchi et al, 1998), MRP4 (Kool et al, 1997; Schuetz, 1999), MRP5 (McAleer et al, 1999), and MRP6 (Kool et al, 1999) mRNA has been observed in various tissues, including the kidney. However, expression of MRP 4 in the kidney has not been observed (Lee et al, 1998). The localization and nephron distribution of these novel MRPs in the kidney are unknown at present. Their roles in the kidney also remain to be elucidated.

Functional properties of organic cation transport system

In the kidney, the organic cation transport system and the organic anion transport system also play physiological and pharmacological roles in the secretion and/or reabsorption of endogenous organic cationic compounds, such as choline, guanidine, monoamine (dopamine, epinephrine, and histamine), cationic drugs (tetraethyl ammonium, cimetidine, quinidine, and procainamide), and cationic xenobiotics (Fig. 1 and Table 4). OCT activity has been observed in renal proximal tubules, distal tubules and collecting ducts (Acara et al, 1979; Mckinney, 1982; Bevan & Kinne, 1990). Functional determinations of OCT were performed using isolated renal tubules (Schali et al, 1983; Smith et al, 1988; Groves et al, 1994), plasma membrane vesicle from renal proximal tubules (Holohan & Ross, 1981; Takano et al, 1984; Takano et al, 1985; Miyamoto et al, 1989; Montrose-Rafizadeh et al, 1989), and stop-flow microperfusion of proximal tubules (David et al, 1995).

Kinsella et al (1979) reported the existence of distinct transport systems at the basolateral and brush-border membranes based on the studies of NMN uptake using the dog kidney. In addition, the valinomycin- and K^+-induced transmembrane potential (inside, negative) enhanced TEA uptake by isolated renal basolateral membrane vesicles, but not by brush-border membrane vesicles (Takano et al, 1985). Functional properties of OCT is extensively studied using isolated brush-border membrane vesicles (Holohan & Ross, 1981; Takano et al, 1984; Montrose-Rafizadeh et al, 1989) and cultured renal epithelial cells (Saito et al, 1992; Ohtomo et al, 1996; Tomita et al, 1997). Many data revealed that the transport system of these membranes is mediated by an electroneutral H^+/organic cation antiporter energized by the transmembrane H^+ gradient. The existence of an additional H^+/organic cation antiporter, more specific for guanidine, was demonstrated in rabbit renal brush-border membranes (Miyamoto et al, 1989). This antiporter was not inhibited by TEA and NMN.

Several endogenous organic cations, such as monoamines and choline, undergo renal tubular reabsorption as well as secretion. Dopamine and epinephrine have been suggested to participate in the reabsorption of Na^+ along the lower nephron segment. This reabsorption mechanism in the brush border membranes could be responsible for nephrotoxicity.

Molecular structure of organic cation transporter

In 1994, Grundmann and coworkers (1994) identi-

fied the first member of the organic cation transporter family, called OCT1, using the expression cloning method. Rat OCT1 is a 556-amino-acid protein with 12 putative transmembrane domains (Fig. 2). Other OCTs have 551-593 amino acid residues. Hydrophobicity analysis predicted that all OCT proteins share a large hydrophilic loop between transmembrane domains 1 and 2. All OCTs have several N-glycosylation sites and protein kinase C sites. Transport studies using isolated rabbit renal proximal tubules (Hohage et al, 1994) and a cultured cell line (Busch et al, 1996) revealed the modulation of organic cation transport by phorbol esters. It is, however, unclear at present, which phosphorylation sites are actually involved in the regulation of organic cation transport by PKC. In addition, studies on the regulatory role of protein kinase A, casein kinase II, and tyrosine kinase have also not yet been performed.

Molecular identification of organic cation transporter

OCT1: OCT1 has been characterized functionally using Xenopus oocytes and cultured cell lines (Table 4) (Grundemann et al, 1994; Zhang et al, 1997; Hohage et al, 1998). When expressed in oocytes, rOCT1 stimulated uptake of TEA, which was inhibited by diverse organic cations (Grundemann et al, 1994). Electrophysiological investigations using rOCT1-expressing oocytes under voltage-clamped conditions revealed that positive inward currents were induced when TEA, NMN, choline, dopamine, or MPP was added to the bath medium, indicating that the rOCT1-mediated cation transport is electrogenic (Busch et al, 1996). Human OCT1 with 78% identity with rOCT1 was also identified from liver. Human OCT1 also mediated the transport of TEA, NMN, MPP, decynium-22, tetrapentylammonium, and quinine. Using the polarized cell line TEA uptake by rOCT1 transfectants grown on microporous filters was markedly enhanced when TEA was added to the basolateral bath medium, but not when it was added to the apical medium (Urakami et al, 1998). TEA uptake was decreased by acidifying the medium pH, suggesting that rOCT1-mediated uptake was pH sensitive.

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. The rOCT1 protein was localized to the basolateral membranes of S1 and S2 segments of proximal tubules and the small intestine and liver (Koepsell et al, 1999). In hOCT1, there is a distinct species difference in mRNA expression, which is observed only in the human liver (Gorboulev et al, 1997).

OCT2: Using hybridization techniques, rat OCT2 cDNA was identified from the rat kidney (Table 4) (Okuda et al, 1996). When rOCT was expressed in oocytes, uptake of TEA was suppressed by the replacement of Na^+ with K^+, indicating that the transport of substrate by rOCT2 was membrane potential-dependent (Okuda et al, 1996). Using the polarized cell line the mode of TEA uptake by rOCT2 transfectants was similar to that by rOCT1 (Urakami et al, 1998). Recently, Urakami et al reported that there is gender difference in transport activity for TEA. Slices and isolated basolateral membrane vesicles of the male rat kidney showed a higher TEA uptake level than that of the female rat kidney (Urakami et al, 1999). rOCT2 showed the 67% identity with rOCT1. Human OCT2 was also identified and characterized. It showed 80% identity with rOCT2. The substrate specificity study of hOCT2 showed that it transports dopamine, norepinephrine, epinephine, 5-hydroxytryptamine, and amantadine (Busch et al, 1998).

In the case of northern blot analysis and RT-PCR, the rOCT2 and hOCT2 mRNAs were detected predominantly in the kidney, but not in the liver, lung, or intestine (Okuda et al, 1996; Gorboulev et al, 1997). hOCT2 was detected in distal convoluted tubules. The detailed localization on the cell membrane and nephron distribution in the kidney is not yet known.

OCT3: An additional member of the OCT family was identified and characterized, designated as OCT3, from the rat placenta (Table 4). rOCT3 is 551-amino-acid protein with 12 putative membrane-spanning domains and shows 48% identity with rOCT1 (Kekuda et al, 1998). When expressed in HeLa cells and Xenopus oocytes, rOCT3 showed uptake of TEA and guanidine, which was inhibited by MPP. Electrophysiological investigations using rOCT3-expressing oocytes under voltage-clamped conditions revealed that the rOCT3-mediated TEA uptake evoked a potential-dependent inward current, which was markedly influenced by the extracellular pH. Mouse and human orthologs were also isolated from the mouse and human placenta (Wu et al, 2000). The amino acid sequence of human OCT3 is identical to that of the

Table 4. Molecular characteristics of organic cation transporters in the kidney

Trnasporter (HGNC name)	Species	Accession	Chromosome	Tissue distribution	Substrate
OCT1 (SLC22A1)	Human	U77086	6q26	liver	TEA, NMN, choline,
	Rat	X78855		liver, kidney, small intestine	dopamine, MPP
	Mouse	AF010259			
OCT2 (SLC22A2)	Human	X98333	6q26	kidney, spleen,brain, placenta	TEA, choline, dopamine, NMN, MPP
	Rat	D83044		kidney, brain	
	Mouse	AJ006036			
OCT3 (SLC22A3)	Human	AF078749	6q26-27	liver, placenta, heart, brain, kidney, lung, small intestine	TEA, guanidine
	Rat	AF055286		placenta, heart, brain, kidney, small intestine	
	Mouse	AF078750		placenta, kidney, brain	
OCTN1 (SLC22A4)	Human	AB007448	5	kidney, bone marrow, spleen, etc.	L-carnitine, TEA, verapamil, quinidine
	Rat	AF169831			
	Mouse	AF111425			
OCTN2/CT1 (SLC22A5)	Human	AF057164	5q31	kidney, placenta, skeletal muscle, pancreas	L-carnitine, TEA
	Rat	AB017260		ubiquitous	L-carnitine
	Mouse	AF110417		ubiquitous	

The substrate specificities were examined for each rat clone.
TEA, tetraethyl ammonium bromide; NMN, N1-methylnicotinamide; MPP, 1-methyl-4-phenylpyridinium.

human EMT (extraneuronal monoamine transporter) recently cloned by Grundemann et al. (1998). These orthologs of mouse and human showed 98% and 86% identity with rat OCT3, respectively. The mouse OCT3 gene is comprised of 11 exons and 10 introns. The Orthologs also showed uptake of TEA, guanidine, and MPP. Northern blot analysis indicated that rOCT3 mRNA was detected most abundantly in the placenta and moderately in the intestine, heart, and brain. The expression level of rOCT mRNA was comparatively low in the kidney and lung, and it was not detected in the liver. Human OCT3 mRNA was strongly expressed in the liver, placenta, kidney and skeletal muscle. The expression was comparatively weak but readily detectable in the lung, heart, and brain. Wu et al. showed the distributional pattern of mouse OCT3 mRNA in the kidney by in situ hybridization. Abundant expression of mOCT3 mRNA was detected in the cortical region with little or no expression in the medulla. The expression was detected specifically in proximal and distal convoluted tubules and within Bowman's capsule but was absent in the glomerulus (Wu et al, 2000). The localization and nephron distribution of OCT3 in the kidney is unknown at present.

OCTN1, OCTN2, and carnitine transporter (CT1)

During a search for homology with OCT transporters, two additional members of the OCT gene family, designated hOCTN1 (Tamai et al, 1997) and hOCTN2 (Tamai et al, 1998; Wu et al, 1998) were cloned from the human fetal liver and rat intestine (Table 4). hOCTN1 is a 551-amino-acid protein with 11 putative transmembrane domains. When expressed in HEK293 cells, hOCTN1 mediated the saturable

and pH-dependent uptake of TEA with higher activity at neutral and alkaline pH than at acidic pH (Yabuuchi et al, 1999). Efflux of TEA by hOCTN1 was also dependent on the acidic external medium pH.

hOCTN2 was identified as a homologue of hOCTN1 from the human kidney. hOCTN2 is a 557-amino-acid protein with 76% similarity to hOCTN1 (Tamai et al, 1998; Wu et al, 1998). When expressed in HEK293 cells, hOCTN2 mediated the uptake of L-carnitine in a sodium dependent manner. It also mediated the uptake of TEA and guanidine (Tamai et al, 1998). Two mutations that result in amino acid substitution in OCTN2, P478L in human and L352R in mouse, have been reported (Wu et al, 1999). Interestingly, although these mutations resulted in the complete loss of the carnitine transport function, the M352R mutant appears to have lost the organic cation transport function, whereas the P478L mutant had higher organic cation transport activity than the wild-type transporter.

Based on the search for OAT isoforms, we identified a clone with relatively ubiquitous tissue distribution and named it carnitine transporter 1 (CT1) (Sekine et al, 1998). CT1 mediated the high-affinity transport of L-carnitine (Km=25 μM) in a partially sodium-dependent manner. Octanoylcarnitine, acetylcarnitine, and γ-butyrobetaine potently inhibited the CT1-mediated transport of carnitine.

hOCTN1 mRNA was found to be abundant in the kidney, trachea, bone marrow, fetal liver and several human cancer cell lines, but not in the adult liver. hOCTN2 is strongly expressed in the kidney, trachea, spleen, bone marrow, skeletal muscle, heart and placenta in adult human. From the tissue distribution and homology of amino acid sequence between hOCTN2 and CT1, hOCTN2 appears to be a human homologue of CT1. The nephron distribution and the role of OCTNs and CT1 in renal secretion of organic cations remain unknown.

MDR1/P-glycoprotein

MDR1 is a member of the ABC multidrug transporter superfamily (Table 3). MDR1 actively extruded drugs with diverse structures, such as vinca alkaloids, steroids, cyclosporines, tacrolimus, anthracyclines, and miscellaneous hydrophobic cations, from the cells (Ford & Hait, 1990). Immunohistochemical analysis using the monoclonal antibody in normal human tissues revealed that the MDR1 protein was found in liver, pancreas, kidney, colon, and jejunum. MDR1 was particularly found to be concentrated on the apical surface of epithelial cells of the proximal tubules in the kidney (Thiebaut et al, 1987).

CONCLUSION

The past few years have witnessed great advances in our knowledge of the molecular pharmacology of renal organic ion transport. This organic ion transport system is also important as a drug transport system. In the field of pharmacology, the renal organic ion transport system is closely associated with pharmacokinetics, as it secretes numerous anionic or cationic drugs. Molecular techniques enabled us to obtain much information about the identity of the organic ion transporters. There is still a large gap in our knowledge about the contribution of individual transporters to the membrane steps involved in tubular secretion of specific substrates. The availability of specific antibodies against cloned carrier proteins will enable us to obtain more detailed and fundamental insights into membrane translocation as well as transepithelial secretion. Particularly in OAT and OCT, the mechanism of excretion of organic anions and cations into the primary urine is unknown. Understanding the detailed transport mechanism and distribution of each cloned transporter in the kidney will give us much information about renal drug clearance, drug-drug interaction, drug targeting to the kidney, and xenobiotic- or drug-induced nephropathy.

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