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Expert Review

Organic Anion Transporters of the SLC22 Family: Biopharmaceutical, Physiological, and Pathological Roles

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Abstract. The human organic anion transporters OAT1, OAT2, OAT3, OAT4 and URAT1 belong to a family of poly-specific transporters mainly located in kidneys. Selected OATs occur also in liver, placenta, and brain. OATs interact with endogenous metabolic end products such as urate and acidic neutrotransmitter metabolites, as well as with a multitude of widely used drugs, including antibiotics, antihypertensives, antivirals, anti-inflammatory drugs, diuretics and uricosurics. Thereby, OATs play an important role in renal drug elimination and have an impact on pharmacokinetics. In this review we focus on the interaction of human OATs with drugs. We report the affinities of human OATs for drug classes and compare the putative importance of individual OATs for renal drug excretion. The role of OATs as sites of drug–drug interaction and mediators cell toxicity, their gender-dependent regulation in health and diseased states, and the possible impact of single nucleotide polymorphisms are also dealt with.

KEY WORDS: drug transport; kidney; OAT1; OAT2; OAT3; OAT4; URAT1.

INTRODUCTION

The organic anion transporters (OATs) of the SLC22 gene family (1) are characterized by a remarkably broad substrate specificity: they handle small, amphiphilic organic anions of diverse chemical structures, uncharged molecules, and even some organic cations (2). Typically, substrates of OATs have a molecular weight of up to 400–500 Da and are classified as "type I" organic anions (3,4). Given the broad specificity, it is of no surprise that organic anion transporters interact with many commonly used anionic drugs such as ß-lactam antibiotics, antivirals, ACE inhibitors, diuretics, NSAIDs etc. (2). Since OATs are typically found at boundary epithelia, these transporters play an important role in distribution and excretion of drugs. Moreover, OATs can be the site of drug–drug interactions during competition of two or more drugs for the same transporter, and mediate cell damage by transporting cytotoxic compounds.

OATs do not directly utilize ATP hydrolysis for energetization of substrate translocation. Most, if not all, members of the OAT family operate as anion exchangers, i.e., they couple the uptake of an organic anion into the cell to the release of another organic anion from the cell. Thereby, OATs utilize existing intracellular > extracellular gradients of anions, e.g., α-ketoglutarate, lactate and nicotinate, to drive uphill uptake of organic anions against the inside negative membrane potential. In kidney proximal tubules, OATs are functionally coupled to Na^+-driven mono- and dicarboxylate transporters that establish and maintain the intracellular > extracellular gradients of lactate, nicotinate, and α-ketoglutarate (see later: "Arrangements of OATs and driving systems" and Fig. 2).

Since 1997, several organic anion transporters have been cloned. A number of recent reviews summarize the present knowledge on overall characteristics (3–5), substrate specificity and drug transport (2,6–10), regulation (11), cell toxicity (12), clustering of OATs with scaffolding proteins (4,13), and genetic organization (14). This review shall focus on the physiological and pharmacological roles of human OATs.

CLONING, MOLECULAR PROPERTIES, LOCALIZATION AND FUNCTION OF INDIVIDUAL OATS

Figure 1 shows a dendrogram of all functionally characterized Organic Anion Transporters that belong to the solute carrier family SLC22. Not included are those cloned members for which there is yet no full publication on their characteristics. In describing the OATs, we shall use prefixes (h, mk, p, rb, r, f) to indicate the species (human, monkey, pig, rabbit, rat, flounder) from which the respective transporter was cloned, e.g. hOAT1 for human OAT1.

OAT1

This was the first organic anion transporter to be cloned from rat (15,16), mouse (17), and flounder kidneys (18). Later on, the orthologues from human (19–21), monkey (22), pig (23), rabbit (24), and *C. elegans* (25) were cloned. The

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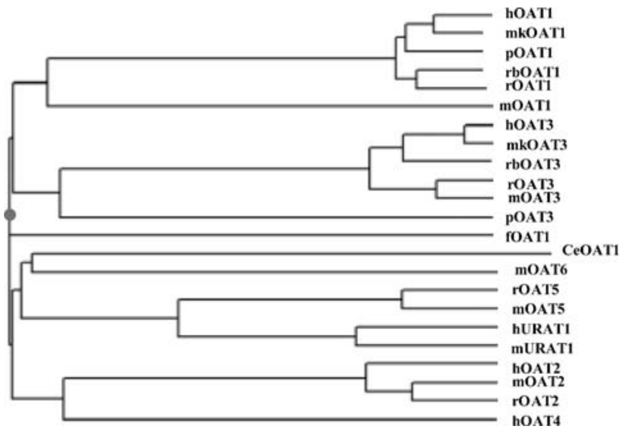

Fig. 1. Phylogenetic relation of cloned and functionally characterized organic anion transporters. The phylogram was constructed using PhyloDraw software http://pearl.cs.pusan.ac.kr/phylodraw.

organic anion transporter from flounder kidney turned out to be functionally an intermediate between, or a precursor of, OAT1 and OAT3 (26). Likewise, it is not clear whether the *C. elegans* "OAT1" is an orthologue of the mammalian OAT1. Fig. 1 would rather suggest a distant relation to mOAT6.

The gene for hOAT1, SLC22A6, is located on chromosome 11q12.3 (20,27), being paired with the gene for OAT3 (28). The mammalian OAT1s consist of 545–551 amino acids, and secondary structure algorithms predict 12 transmembrane helices with the N- and C-termini located at the cytosolic side of the plasma membrane. In man, a longer splice variant with 563 amino acids and two shorter, nonfunctional splice variants were found (20,29). The large extracellular loop between transmembrane helix (TM) 1 and TM2 carries several glycosylation sites, and the intracellular loop between TM6 and TM7 and the C-terminus harbour several consensus sequences for phosphorylation by protein kinases. The glycosylation of human and mouse OAT1 is important for proper shuttling of newly synthesized transporters to the cell membrane (30). The role of phosphorylation sites is unclear: canonical protein kinase C consensus sites were not involved in the down-regulation of human and mouse OAT1 (31,32), and the sites for casein kinase II, protein kinase A and tyrosine kinases have not been studied so far.

Amino acid residues important for transport function were analysed by site-directed mutagenesis. In flounder OAT, the cationic amino acid residues lysine at position 394 (TM 8) and arginine at position 478 (TM11) are involved in binding and translocation of dicarboxylates (33). In hOAT1, the arginine at position 466 (TM11) appears to be involved in the interaction with dicarboxylates and with chloride which activates this transporter (unpublished results).

Immunohistological studies revealed the expression of OAT1 at the basolateral membrane of proximal tubule cells in human (20,34), rat (35–37), and mouse (38) kidneys. Besides kidneys, human OAT1 has been shown to be located at the choroid plexus (39), and recent studies on mouse brain revealed mOAT1 expression in neurones of cortex and hippocampus (38).

Endogenous compounds. OAT1, together with OAT3, is responsible for the first step of renal organic anion secretion, the uptake of organic anions from the blood across the basolateral membrane into proximal tubule cells. The uptake occurs in exchange for intracellular α-ketoglutarate (see also Fig. 2). OAT1 interacts with a vast number of endogenous and exogenous organic anions. The transport of the following radioactively labeled, endogenous compounds has been demonstrated: the metabolic intermediate α-ketoglutarate (15,21), the local hormones prostaglandin E_2 and $F_{2\alpha}$ (15,40), the second messengers cAMP and cGMP (15), the vitamin folate (41), and the purine breakdown product urate (15,42). Several other endogenous compounds inhibit OAT1, e.g., the hormones corticosterone and dehydroepiandrosterone sulfate (43,44), the vitamin nicotinate (45), the purine metabolites xanthine and hypoxanthine (45), and acidic metabolites of the neurotransmitters norepinephrine (vanillinemandelate), dopamine (3,4-dihydroxyphenylacetate, homovanillate), 5-hydroxytryptamine (5-hydroxyindoleacetate), and of cerebral tryptophan metabolism (quinolinate, kynurenate) (38,39). The interaction of OAT1 with neurotransmitter metabolites strongly suggests that OAT1 is responsible for both removal of these metabolites from the brain and for renal excretion.

Recently, an OAT1 knockout mouse has been generated (46). The mice were normal and fertile. The kidneys were histologically unchanged, had a normal GFR, salt and water excretion. *p*-Aminohippurate clearance and the excretion of 3-hydroxybutyrate, 3-hydroxyisobutyrate, 3-hydroxypropionate, benzoate, 4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, 4-hydroxyphenylacetate and *N*-acetylaspartate were decreased, indicating that these substances occur endogenously as metabolites and are secreted through OAT1. For some of these substances, K_i values were determined: 4-

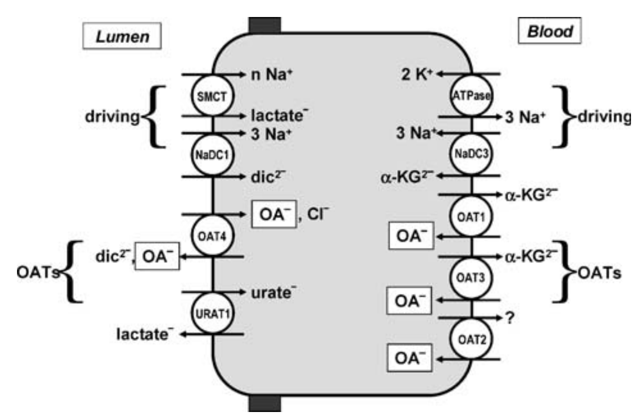

Fig. 2. Localization of organic anion transporters in a human renal proximal tubule cell. The lumen (urine or apical side) is on the *left hand side*, and the blood (interstitium or basolateral side) at the *right hand side*. The upper transporters SMCT and NaDC1 in the apical, and Na^+,K^+-ATPase and NaDC3 in the basolateral membrane are collectively driving the organic anion transporters OAT4 and URAT1 in the apical, and OAT1 and OAT3 in the basolateral membrane. The driving ion for OAT2 is unknown. Abbreviations: *α-KG*$^{2-}$ α-ketoglutarate; *dic*$^{2-}$ dicarboxylate (succinate, α-ketoglutarate); *NaDC* sodium dicarboxylate cotransporter; *OA*$^-$ organic anion/anionic drug; *OAT* organic anion transporter; *SMCT* sodium monocarboxylate cotransporter; *URAT* urate transporter.

hydroxyphenylpyruvate (56 μM), benzoate (253 μM), 4-hydyroxyphenyllactate (390 μM), *N*-acetylaspartate (841 μM), 3-hydyroxybutyrate (3.3 mM) (46). Thus, OAT1 contributes to renal excretion of various metabolites, but normal life in mice is possible without this transporter, probably because OAT3 can take over the task.

Exogenous compounds. Numerous drugs have been tested as possible substrates of OAT1. Either transport of radiolabelled drugs was demonstrated or inhibition of uptake of a prototypical labelled substrate, *p*-aminohippurate (PAH), by unlabelled drugs was shown. Table I gives some examples of drugs that have been found to interact with human OAT1. Data on drug transport by rodent OAT1 are not included (for review see (2)). The following short paragraphs provide some additional information on the interaction of hOAT1 with drug classes selected from Table I.

(a) Antibiotics. Human OAT1 interacted with penicillines, cephalosporines, and tetracyclines. Translocation was only shown for tetracycline (47). However, tetracycline uptake into mouse proximal tubule cells transfected with hOAT1 was hardly greater than uptake into mock cells, suggesting to us that OAT1 does not contibute much to renal tetracycline excretion. All other antibiotics inhibited OAT1 function. Conflicting results exist for benzylpenicillin that inhibited hOAT1 expressed in oocytes (20), but not hOAT1 expressed in HeLa cells (21). For cephalosporines, the reported and later corrected K_i values are: 6.14 mM for cefadroxil; 30 μM for cefamandole; 180 μM for cefazolin; 210 μM for cefoperazone; 3.13 mM for cefotaxime; 230 μM for ceftriaxone (48); 0.74 and 1.25 mM for cephaloridine (48,49); 220 μM for cephalotin (48); and 1.6 mM for cephradine (49), indicating a moderate to low affinity of hOAT1 for these antibiotics (see also Table VI). Since inhibition of PAH uptake by cephalotin was competitive, an interaction of all cephalosporines with the substrate binding and transport site of hOAT1 was assumed (48). Transport of radiolabeled cephalosporines, however, was not yet shown and, hence, transport activity (V_{max}/K_m) and exact contribution of hOAT1 to overall renal excretion remain open.

(b) Antivirals. There is clear experimental evidence for the translocation of antiviral drugs by hOAT1. The K_m values for uptake are: 342 μM for acyclovir (50); between 17.2 and 30 μM for adefovir (26,51–53); between 46 and 58 μM for cidofovir (49,51–53); 896 μM for ganciclovir (50); 22.3 μM for tenofovir (53); and 45.9 μM for zidovudine (50). Acyclovir and ganciclovir are guanosine analogs without a negative charge which may explain the relatively low affinity of hOAT1 for these antivirals. The thymidine analogue zidovudine is also uncharged, but has a relatively high affinity, suggesting that the base moiety and, hence, the ability to form hydrogen bonds, plays a role in determining the interaction with hOAT1. Adefovir, cidofovir and tenofovir are nucleotide analogues and carry negatively charged phosphate groups that probably foster binding to, and translocation by, hOAT1. Importantly, antiviral drugs are nephrotoxic, and the expression of OAT1 renders cells sensitive to these compounds (51). The coadministration of probenecid or NSAIDs, i.e., intended drug–drug interaction, reduced the cytotoxicity of antiviral drugs (54).

(c) H2 antagonists. The blockers of histamine receptor subtype 2, cimetidine and ranitidine, are translocated by

Table I. Examples of Drugs Interacting with Human OAT1

Class	Tested Compounds	Reference
Antibiotics	benzylpenicillin, cefadroxil, cefamandole, cefazolin, cefoperazone, cefotaxime, ceftriaxone, cephaloridine, cephalotin, cephradine, cinoxacin, doxycyclin, minocycline, nalidixate, oxytetracycline, tetracycline[b]	(20,45,47–49)
Antivirals	acyclovir[b], adefovir[b], cidofovir[b], ganciclovir[b], PMEDAP[a,b], PMEG[a,b], tenofovir[b], zalcitabine[b], zidovudine[b]	(26,49–53,180)
H_2 antagonists	cimetidine[b], ranitidine[b]	(55,56,67,68,99,181)
Antihypertensives	captopril, losartan	(45,80)
Cytostatic	methotrexate[b]	(63)
Diuretics	acetazolamide, bumetanide[b], chlorothiazide, cyclothiazide, ethacrynate, furosemide[b], hydrochlorothiazide, methazolamide, trichlormethiazide	(20,21,29,60,80,94,181)
NSAIDs[a]	acetaminophen, acetylsalicylate, diclofenac, diflusinal, etodolac, flufenamate, flurbiprofen, ibuprofen[b], indomethacin[b], ketoprofen[b], loxoprofen, mefenamate, naproxen, phenacetin, phenylbutazon, piroxicam, salicylate, sulindac	(20,21,42,49,54,62–64,80,94,180,181)
Statins	fluvastatin, pravastatin, simvastatin	(45,65,67,139)
Uricosurics; purine metabolism	allopurinol, benzbromarone, probenecid	(20,21,42,45,45,49,51,52, 54,64,66–68,80,94,139,140,146, 147,151,154,179,181–183)

[a] Abbreviations: *NSAID* non-steroidal anti-inflammatory drug; *PMEDAP* 9-(2-phosphonyl-methoxyethyl)-diaminopurine; *PMEG* 9-(2-phosphonyl-methoxyethyl)-guanidine
[b] transport has been demonstrated

hOAT1 (45,55,56). The dependence of cimetidine transport on pH suggested that it is the uncharged form of cimetidine (cim^0) that is substrate of OAT1, whereas the charged form (cim^+) is translocated by renal OCTs (55) and by OAT3 (57–59). Famotidine, another H_2 blocker, was not a substrate of hOAT1, but was translocated by hOAT3 (55).

(d) Diuretics. Loop and thiazide diuretics inhibit from the tubule lumen the salt transporters in the thick ascending limb of Henle's loop and in the distal convoluted tubule, respectively. OATs are involved in drug targeting by secreting diuretics into the tubule lumen. The loop diuretics bumetanide, ethacrynate and furosemide inhibited hOAT1-driven PAH uptake with IC_{50} values (in μM) of 7.6; 29.6; and 18, respectively (60). The corresponding IC_{50} values for the thiazide diuretics chlorothiazide, cyclothiazide, hydrochlorothiazide, and trichlormethiazide were (in μM) 3.8; 84.3; 67.3; and 19.2, respectively (60). Thus, all these diuretics interact with high to moderate affinity with hOAT1. Translocation by OAT1 has been demonstrated so far for bumetanide, although the uptake was much slower than that by OAT3, and for furosemide (60). The transport of the other diuretics by hOAT1, though likely, remains to be demonstrated experimentally.

(e) NSAIDs. Several non-steroidal anti-inflammatory drugs were tested on rodent (61) and human OAT1. With hOAT1 the following IC_{50} values were reported: 639 μM for acetaminophen; 769 μM for acetylsalicylate (62); 4 and 4.6 μM for diclofenac (54,62); 0.85 μM for diflusinal; 50 μM for etodolac; 1.5 μM for flurbiprofen (54); 8 and 55.5 μM for ibuprofen; 3 and 3.83 μM for indomethacin (54,62); 1.3, 1.4 and 4.43 μM for ketoprofen (49,54,62); 27.1 μM for loxoprofen (63); 0.83 μM for mefenamate (62); 5.7 and 5.8 μM for naproxen; 200 and 275 μM for phenacetin (54,62); 19.8, 20.5 and 62.8 μM for piroxicam (54,62,64); 280, 325 and 1,573 μM for salicylate (42,49,62); and 36.2 μM for sulindac (62). Taken together, hOAT1 showed high to moderate affinities for NSAIDs (see also Table VI). Translocation by human OAT1 was directly demonstrated for ibuprofen, indomethacin, and ketoprofen (62). In another study, translocation of ibuprofen and ketoprofen was not found (54). The reason for this discrepancy remains unknown. It is worth noting that most NSAIDs show high plasma protein binding and little renal excretion (62). Thereby, the free plasma concentrations of NSAIDs may be considerably smaller than the IC_{50} values measured *in vitro*, and an interaction between most of these drugs and hOAT1 may not occur *in vivo*.

(f) Statins. The HMG–CoA reductase inhibitors fluvastatin, pravastatin, and simvastatin inhibited hOAT1-mediated PAH transport with IC_{50} values of 26.3; 408; and 73.8 μM, respectively (65). Labeled pravastatin was not transported by hOAT1. In addition, the free plasma concentrations are much lower than the IC_{50} values, making a substantial contribution of hOAT1 to renal excretion of statins unlikely (65).

(g) Uricosurics. The xanthine oxidase inhibitor allopurinol that is used to decrease plasma urate levels, inhibited hOAT1-mediated PAH transport (45). Benzbromarone also decreased OAT1-mediated transport and showed, with an IC_{50} of 4.6 μM, a high affinity for OAT1 (42). Most citations in Table II refer to the inhibitory effect of probenecid on human OAT1. The reported IC_{50} values range between 4.29 and 12.5 μM with an average of 7.94 μM determined in eight publications with different substrates for hOAT1 (42,49,51,54,64,66–68). Benzbromarone and probenecid are uricosuric, i.e., they decrease proximal tubular urate reabsorption and/or increase urate secretion. The inhibition of OAT1 by uricosurics can be explained by assuming that OAT1 physiologically transports urate from the cell to the blood. Benzbromarone and probenecid would then inhibit urate absorption by inhibiting both URAT1 at the apical membrane (see below) and OAT1 in the basolateral membrane, working in series to accomplish urate absorption.

OAT2

With an antibody against an unknown liver canalicular protein, a putative transporter was cloned and named NLT

Table II. Examples of Drugs Interacting with Human OAT2

Class	Tested Compounds	Reference
Antibiotics	cefadroxil, cefamandole, cefazolin, cefoperazone, cefotaxime, ceftriaxone, cephaloridine, cephalotin, chloramphenicol, doxicycline, erythromycin[c], minocycline, oxytetracycline, tetracycline	(47,79)
Antivirals	zidovudine[c], ganciclovir	(47,50)
H_2 antagonists	cimetidine[c], ranitidine[c]	(55)
Cytostatics	5-fluorouracil[c], methotrexate[c], taxol[c]	(71,76)
Diuretics	bumetanide[c], chlorothiazide, cyclothiazide, ethacrynate, furosemide, hydrochlorothiazide, trichlormethiazide	(60,60,76)
NSAIDs[a]	acetaminophen[b], diclofenac[b], ibuprofen, indomethacin[b], ketoprofen, mefenamate, naproxen, phenacetin, piroxicam, salicylate[c], sulindac	(62,76)
Statin	pravastatin	(67)
Uricosurics; purine metabolism	allopurinol[c], probenecid	(73,76)

[a] Abbreviations: *NSAID* non-steroidal anti-inflammatory drug
[b] Conflicting results
[c] Transport has been demonstrated

for novel liver-specific transporter (69). Later on, NLT was recloned from rat liver, expressed, and named OAT2 because of its relation to OAT1 and interaction with organic anions (70). Human (71) and mouse (72) OAT2s have also been cloned and functionally characterized. The gene for human OAT2, SLC22A7, is located on chromosome 6q26, and is not paired with any other OAT gene (28). Human, rat and mouse OAT2 consist of 535–548 amino acids, and secondary structure predictions revealed a membrane topology identical to those of other OATs (12 transmembrane helices).

Immunohistological studies placed human OAT2 at the basolateral membrane of proximal tubules (73). In contrast, in rat kidneys OAT2 appeared to be located at the apical membrane of collecting ducts, and in the thick ascending limb of Henle's loop, but not in proximal tubules (35). In mice, OAT2 was found again in the apical membrane, but in this species in proximal tubules (74). Recently, the immunolocalization of OAT2 was re-evaluated in rat and mice kidneys. In both species, OAT2 was found at the apical (brush-border) membrane of late proximal tubules, i.e., in segment S3 (75). Thus there are species differences with respect to the renal localization of OAT2 in humans and rodents. The subcellular location of OAT2 in the liver, the main site of expression, is unknown.

The transport mode of OAT2 is, in our opinion, not yet resolved. Human OAT2 was reported to transport α-ketoglutarate, but this dicarboxylate did not inhibit OAT2-mediated PAH transport (71). hOAT2 expressed in oocytes transported labelled glutarate (a dicarboxylate with five carbons), but glutarate did not *trans*-stimulate estrone sulfate uptake, when preloaded into the oocytes (76). Instead, the authors proposed that the 4-carbon dicarboxylates succinate and fumarate *trans*-stimulate estrone sulfate uptake, but their data do not support this assumption. Since also for rOAT2 conflicting results exist with regard to the interaction with α-ketoglutarate (see (2)), it is unclear, whether and how OAT2 interacts with dicarboxylates. Defining the driving force requires further experiments, particularly in the light of the different locations (apical membrane in rodent kidneys, basolateral membrane in human kidneys) of OAT2.

Endogenous compounds. Human OAT2 was reported to transport the second messenger cAMP (71); the hormones dehydroepiandrosterone sulfate (DHEAS (76)), estrone-3-sulfate (ES (76)), and prostaglandins E_2 (40,76) and $F_{2\alpha}$ (40,73); the vitamin L-ascorbate (76); and the citric acid cycle intermediate α-ketoglutarate (71). In other publications, hOAT2 did not transport DHEAS (71) and α-ketoglutarate (77). The reason for these discrepancies is unclear.

Exogenous compounds. As compared to OAT1 and OAT3, a limited number of drugs have been tested with OAT2 (see Table II). Again, we focus on the interaction of human OAT2 with drugs.

(a) Antibiotics. OAT2 interacted with cephalosporines, tetracyclines, chloramphenicol and the macrolide erythromycin. Erythromycin (K_m 18.5 μM (78)) and tetracycline (K_m 440 μM (47)) were shown to be transported by hOAT2. The other antibiotics were used as inhibitors of hOAT2-mediated prostaglandin $F_{2\alpha}$ or tetracycline transport. For cephalosporines the following IC_{50} values were found: 6.4 mM for cefradroxil; 430 μM for cefamandole; 5.1 mM for cefazolin; 1.1 mM for cefoperazone; 5.2 mM for cefotaxime; 6.8 mM for ceftriaxone; 2.1 mM for cephaloridine; and 1 mM for cephalotin (79).

(b) Antivirals. Zidovudine (AZT) was transported by hOAT2 with good affinity (K_m 26.8 μM (50)). Ganciclovir was not taken up by hOAT2, but inhibited transport. Acyclovir and valacyclovir were neither transported nor did they inhibit OAT2-mediated transport (50). It appears that OAT2 does not appreciably interact with antiviral drugs.

(c) H2 antagonists. Data on the H2 blocker cimetidine are conflicting. Transport was shown in HEK293 cells expressing hOAT2 (55), and inhibition of transport in rOAT2-expressing LLC-PK1 cells (77). No inhibition was seen, however, with human and rat OAT2 expressed in mouse proximal tubule cells (67). Ranitidine was translocated by hOAT2 (55).

(d) Diuretics. Carbonic anhydrase inhibitors, loop and thiazide diuretics interacted with human OAT2. For bumetanide, either no transport (60) or translocation with high affinity (K_m 7.5 μM (76)) was found. For the other diuretics, IC_{50} values were determined: >5 mM for acetazolamide; 77.5 μM for bumetanide; 2.2 mM for chlorothiazide; 39.2 μM for cyclothiazide; 121 μM for ethacrynate; 603 μM for furosemide; 1 mM for hydrochlorothiazide; and 1.2 mM for trichlormethiazide (60). hOAT2 has an intermediate (bumetanide, cyclothiazide, ethacrynate) to low affinity (furosemide, hydrochlorothiazide, trichlormethiazide, chlorothiazide) for, or shows negligible interaction (acetazolamide) with, diuretics.

(e) NSAIDs. Radiolabelled salicylate, but not acetylsalicylate, indomethacin, ketoprofen and ibuprofen, was transported by hOAT2 (62). Thereby, salicylate uptake was less than twofold the uptake observed in mock cells, indicating a slow transport rate. For several NSAIDs, the half-maximal inhibitor constants were determined: 14.3 μM for diclofenac, 692 μM for ibuprofen, 64.1 μM for indomethacin, 400 μM for ketoprofen, 21.7 μM for mefenamate, 486 μM for naproxen, 1.88 mM for phenacetin, 70.3 μM for piroxicam, and 440 μM for sulindac. The IC_{50} values for acetaminophen, acetylsalicylate, and salicylate could not be determined ($IC_{50} > 2$ mM (62)), suggesting a very low affinity of hOAT2 towards these hydrophilic NSAIDs. All IC_{50} values are considerably higher than those determined for hOAT1 and hOAT3 (see also Table VI).

(f) Cytostatics. Human OAT2 translocated 5-fluorouracil (5-FU), methotrexate and taxol. Thereby, the K_m for 5-FU was 53.9 nM, and that for taxol 143 nM, indicating a very high affinity of OAT2 for these compounds (76). A K_m for methotrexate uptake was not determined.

OAT3

The organic anion transporter 3 was cloned from human (58,80), monkey (22), pig (81), rabbit (59), rat (82) and mouse (83) kidneys. The human OAT3 gene named SLC22A8 is paired with that of OAT1 and located on chromosome 11q12.3 (28,58). Human OAT3 is mainly expressed in kidneys, and to a lesser extent in the brain (58). In rats, more message for rOAT3 was found in liver than in kidneys and brain (82), suggesting species differences. The mammalian OAT3 proteins consist of 536–542 amino acids, arranged in 12 transmembrane helices and with a large extracellular loop between TM1 and TM2, and a large intracellular loop between TM6 and TM7 carrying potential phosphorylation sites for regulation by protein kinases (84). Protein kinase C activation led to an inhibition of rOAT3 probably by induction of internalization (85). In rOAT3, two basic residues, lysine 370 (TM8) and arginine 454 (TM11) are important for anion binding and transport (57). In addition, tryptophan 334 and tyrosine 342 in TM7, and phenylalanine 362 in TM8, are involved in the interaction with substrates (86).

Immunohistochemistry revealed the location of OAT3 at the basolateral membrane of human (34,58), rat (35,36,87,88), and mouse (38) renal proximal tubules. In rats, OAT3 was also found in several other nephron segments including thick ascending limb of Henle's loop, distal convoluted tubule, and collecting ducts (35,36,87). OAT3 in proximal tubules is involved in organic anion secretion, but the physiological and pharmacological roles of OAT3 in deeper nephron segments are presently not clear. The message for OAT3 outweighed that for OAT1 and, by far, those of OAT2 and OAT4 (34). Based on message abundance, OAT3 is the predominant organic anion transporter in the human kidney. Besides kidneys and liver, OAT3 was also found in human choroid plexus (39) and in rat cerebral capillaries (89).

Endogenous substrates. OAT3 is involved in the uptake of organic anions from the blood across the basolateral cell membrane into the proximal tubule cells. Organic anion uptake through OAT3 is coupled to the efflux of α-ketoglutarate (90,91). Human OAT3 was reported to transport the second messenger cAMP (58); the hormones cortisol (92), prostaglandins E_2 and $F_{2\alpha}$ (40,58); the conjugated hormones dehydroepiandrosterone sulfate (DHEAS (58)), estrone sulfate (ES (22,26,58,66,86,90,93,94)) and estradiol-17ß-glucuronide (58); the bile salt taurocholate; and the purine metabolite urate (58). The ability to transport corticosterone, ES, estradiol-17ß-glucuronide, and taurocholate distinguishes OAT3 from OAT1. Inhibition of hOAT3-mediated transport was shown for cholate (58), melatonin, and several anionic neurotransmitter metabolites (39). The physiological function of renal OAT3 appears to be the secretion of steroid hormones, their conjugates, and of prostaglandins. OAT3 expressed in brain and kidneys most probably cooperate in the removal of anionic neurotransmitter metabolites (95).

OAT3 knockout mice turned out to be normal and fertile (96). They did not show any gross organ abnormalities and, in particular, kidneys, liver and brain were unchanged. The uptake of taurocholate, estrone sulfate and bromosulfophthalein (BSP) into kidney slices from knockouts was reduced, as was the uptake of fluorescein into cells of the choroid plexus. Thus, a normal development in mice is possible without OAT3 which may be due to the fact that OAT1 provides a backup system.

Exogenous compounds. Numerous drugs have been tested with human OAT3 (see Table III). For interaction of rodent OAT3 with drugs see (2).

(a) Antibiotics. Human OAT3 transported labelled benzylpenicillin with a K_m of 52.1 μM (22). This high affinity was also found for monkey (49.2 μM (22)), rat (82.8 μM (97)), and mouse (40.0 μM (98)) OAT3, suggesting that OAT3 is the main player in renal benzylpenicillin excretion in all species. Tetracycline was also transported by hOAT3 with a K_m of 566 μM (47). OAT3-mediated ES transport, however, was not inhibited by various tetracyclines, leaving open whether this transporter plays any role in tetracycline excretion. Cephalosporines were tested as inhibitors of ES uptake, and the following IC_{50} values were found: 8.6 mM for cefadroxil, 46 μM for cefamandol, 550 μM for cefazolin, 1.9 mM for cefoperazone, 290 μM for cefotaxim, 4.4 mM for ceftriaxone, 630 μM for cephalexin, 2.5 mM for cephaloridine, and 40 μM for cephalotin (48).

Table III. Examples of Drugs Interacting with Human OAT3

Class	Tested Compounds	Reference
Antibiotics	benzylpenicillin[b], cefadroxil, cefamandol, cefazolin, cefoperazone, cefotaxim, ceftriaxone, cephalexin, cephaloridine, cephalotin, tetracycline[b]	(22,47,48)
Antivirals	acyclovir, ganciclovir, valacyclovir[b], zidovudine[b]	(50)
H_2 antagonists	cimetidine[b], famotidine[b], ranitidine[b]	(22,55,57,58,67,68,94,99,181)
Antiepileptic	valproate	(95)
Cytostatics	azathiopurine, methotrexate[b]	(58,94,181,184)
Diuretics	acetazolamide, bumetanide, chlorothiazide, cyclothiazide, ethacrynate, furosemide[b], hydrochlorothiazide, methazolamide, trichlormethiazide	(58,60,94,181)
NSAIDs[a]	acetylsalicylate, diclofenac, flufenamate, ibuprofen[b], indomethacin[b], ketoprofen[b], loxoprofen, mefenamate, naproxen, phenacetin, phenylbutazone, piroxicam, salicylate[b], sulindac	(58,62–64,94,181)
Statins	pravastatin[b], simvastatin	(65,67,139)
Uricosuric	probenecid	(55,58,64,66,68,94,139,154,181,185)

[a] Abbreviations: *NSAID* non-steroidal anti-inflammatory drug
[b] Transport has been demonstrated

(b) Antivirals. Valacyclovir and zidovudine (AZT) were taken up by mouse proximal tubule cells expressing hOAT3 (50). The K_m for zidovudine (145.1 μM) is higher that than of hOAT1 (45.9 μM) (50). Since the V_{max}/K_m ratios for hOAT1 and hOAT3 were identical, both transporters could contribute to renal zidovudine excretion. In contrast to OAT1, adefovir (26) and ganciclovir (50) were not transported by hOAT3, and valacyclovir not by hOAT1 (50), indicating different structural requirements of OAT1 and OAT3 for the transport of antivirals.

(c) H2 antagonists. hOAT3 interacted with the H2 receptor blockers cimetidine, famotidine, and ranitidine. Unlabeled cimetidine inhibited hOAT3-mediated transport with IC_{50} values ranging between 43.2 and 92.4 μM (67,68,99), and labeled cimetidine was taken up with K_m values between 40 and 149 μM (22,55,57]. Hence, there is no doubt that OAT3 in general translocates cimetidine which is either uncharged (cim^0) or an organic cation (cim^+). The interaction with organic cations was long considered as a specific feature of OAT3, but meanwhile it is clear that also OAT1 can interact with cimetidine (56). It appears, however, that hOAT1 has a lower affinity for cimetidine (IC_{50} 492 μM (67)). Famotidine and ranitidine were also translocated by hOAT3 (55,99). The K_m values for uptake were 124 μM for famotidine and 234 μM for ranitidine (55), and comparable values were found for monkey and rat OAT3 (55,100). Rat OAT3 showed a much lower V_{max}/K_m ratio for famotidine than hOAT3. Although V_{max} depends on the level of protein expression these data tentatively explain why probenecid inhibits renal famotidine excretion in humans (through inhibiting OAT3), but not in rats (low famotidine transport by OAT3; no effect of probenecid on famotidine transport by OCT1). hOAT1 transported little ranitidine and no famotidine (55), indicating a general preference of H_2 antagonists for OAT3.

(d) Diuretics. Carbonic anhydrase inhibitors (acetazolamide, methazolamide), loop diuretics (bumetanide, ethacrynate, furosemide) and thiazides inhibited OAT3-mediated transport. For bumetanide and furosemide, transport by hOAT3 was shown directly (60). The K_m for bumetanide uptake was 1.59 μM and indicated a very high affinity of hOAT3 for this diuretic. In inhibition experiments, bumetanide, ethacrynate and furosemide exhibited IC_{50} values of 0.75, 0.58, and 7.31 μM, respectively (60), supporting the notion that hOAT3 has a high affinity for these compounds. The IC_{50} values for chlorothiazide, cyclothiazide, hydrochlorothiazide, and trichlormethiazide (65.3; 27.9; 942; 71.2 μM, repectively) and acetazolamide and methazolamide (816; 97.5 μM) were generally higher than those for loop diuretics (60).

(e) NSAIDs. Radiolabeled indomethacin, salicylate, ketoprofen, and ibuprofen, but not acetylsalicyate, were transported by hOAT3 (58,62). For indomethacin and ketoprofen, uptake into hOAT3-expressing cells was only slightly higher than that into mock cells, indicating low transport rates. When NSAIDs were tested as inhibitors of hOAT3-mediated estrone sulfate uptake, the following IC_{50} values were obtained: 717 μM for acetylsalicylate, 50 μM for salicylate, 7.78 μM for diclofenac, 6 μM for ibuprofen, 0.61 μM for indomethacin, 5.98 μM for ketoprofen, 0.78 μM for mefenamate, 4.67 μM for naproxen, 19.4 μM for phenacetin, 2.52 μM for piroxicam, and 3.62 μM for sulindac (62). In other studies, IC_{50} values of 4.88 μM for piroxicam (64) and 8.7 μM for loxoprofen (63) were found. Acetaminophen was reported to either inhibit (76) or not to inhibit hOAT3 (62). The reason for this discrepancy is not known. In comparison, the above mentioned IC_{50} values are equal or smaller than those determined for hOAT1, and considerably smaller than those for hOAT2 and hOAT4, indicating that hOAT3 has the highest affinity for NSAIDs (see also Table VI).

(f) Uricosurics. Except for probenecid that was used as an inhibitor of OAT3, no uricosurics have been tested on hOAT3 to our knowledge.

OAT4

The organic anion transporter 4 is the only transporter that is specific for human. It was cloned from a kidney library and found to be expressed in kidneys and in placenta (101). The gene SLC22A11 is located on chromosome 11q13.1 and is paired with the gene for URAT1 (28). The OAT4 protein consists of 550 amino acids, and shows all structural properties typical of OATs (12 transmembrane helices, intracellular N- and C-terminus, long extracellular loop between TM1 and TM2 with glycosylation sites; long intracellular loop between TM6 and TM7 with phosphorylation sites). The glycosylation is important for proper targeting of OAT4 to the membrane, and the type of carbohydrate residues has an influence on the affinity towards estrone sulfate (102). The glycine residues G241 and G400 in TM5 and TM8 are also important for targeting, and replacing these glycines with short chain amino acids in mutant OAT4 decreased the affinity for estrone sulfate (103). Likewise, OAT4 mutants with replacement of several histidine residues showed a reduced appearance at the plasma membrane (104), indicating that several amino acids are important for proper folding and trafficking.

Immunohistologically OAT4 was detected in the apical membrane of proximal tubule cells (105). OAT4 interacts through the C-terminal three amino acids threonine–serine–leucine forming a PDZ motif with the apically located scaffolding proteins NHERF1 and PDZK1 (106,107). It is most probably this interaction that directs OAT4 to, and maintains it in, the apical membrane. Due to its localization, OAT4 is either involved in the absorption of organic anions from the ultrafiltrate, or in the secretion of organic anions that were accumulated in the cell by OAT1 and OAT3. In the placenta, OAT4 may allow for the release of potentially toxic compound from the fetus towards the mother, and also deliver sulfated precursors from the mother for placental estrogen synthesis (108).

Endogenous substrates. OAT4 appears to have a high affinity for the conjugated hormone estrone-3-sulfate (ES) with K_m values for uptake of 1.01 (101) and 6.0 μM (102). Dehydroepiandrosterone sulfate was taken up by OAT4 with a K_m of 0.63 μM (101). Estrone (102) and 17ß-estradiol-3-sulfate, but not ß-estradiol and 17ß-estradiol-3-D-glucuronide, inhibited OAT4-mediated ES uptake (101,102). Together with other results on conjugated compounds (101) it appears that OAT4 transports sulfated, but not glucuronidated hormones. OAT4 exhibited a very high affinity also towards prostaglandin E_2 (K_m 0.15 μM) and prostaglandin $F_{2\alpha}$ (K_m 0.69 μM) (40). Further endogenous anions that inhibited OAT4 were octanoate (105), succinate (109), cholate, taurocholate (101), and urate (110).

Recently, evidence was provided for an OAT4-mediated organic anion/glutarate exchange (111). The endogenous dicarboxylates succinate and α-ketoglutarate were not tested. From our own studies it appeared that OAT4 is an asymmetric carrier: it transports glutarate and *p*-aminohippurate only in the outward direction, i.e., from the cell into the tubule lumen, probably by exchanging them against extracellular (luminal) chloride ions. Estrone sulfate and urate are taken up by OAT4 in exchange for glutarate and, possibly, hydroxyl ions (112). It looks, therefore, that OAT4 provides an exit for organic anions into the lumen (secretion) and an entry for urate and estrone sulfate into the proximal tubule cell (absorption).

Exogenous compounds. Table IV shows examples of drugs interacting with human OAT4. The number of drugs listed is smaller than for OAT1 and OAT3 because not so many drugs were tested, and OAT4 did not transport, or was not inhibited by, a number of test agents.

(a) Antibiotics. Uptake has only been shown for labelled tetracycline. Transport was saturable and exhibited a K_m of 122.7 μM (47). Thereby, OAT4 appears to have a higher affinity for tetracycline than OAT1 (very weak transport), OAT2 (K_m 440 μM), and OAT3 (K_m 566 mM) (47). Unlabeled oxytetracycline, minocycline and doxycycline, however, did not decrease ES uptake by OAT4. Since only 50 μM of these tetracyclines were used in competition experiments (47), it remains open whether OAT4 interacts with these compounds. Benzylpenicillin and various cephalosporines inhibited OAT4-mediated transport of ES and other substrates (48,101,105,113). The IC_{50} values were determined on mouse proximal tubule cells expressing OAT4: 1.14 mM for cefamadol, 1.74 mM for cefazolin, 2.8 mM for cefoperazone, 6.15 mM for cefotaxim, 2.38 mM for ceftriaxon, 3.63 mM for cephaloridine, and 200 μM for cephalotin (48). Taken together, OAT4 has a much lower affinity for cephalosporine uptake than has OAT1. If OAT4 has a similarly low affinity for cephalosporines at the cytosolic side, it may contribute to the nephrotoxic effect of cephaloridine, which would then be avidly taken up into proximal tubule cells by OAT1, but released slowly through OAT4.

(b) Antivirals. When radiolabeled acyclovir, valacyclovir, ganciclovir and zidovudine were added to OAT4-expressing tubule cells, only zidovudine (AZT) showed an uptake greater than that into non-expressing control cells, and a K_m of 152 μM was determined (50). This K_m is similar to that of hOAT3 (145 μM), but higher than those for hOAT1 (45.9 μM) and hOAT2 (26.8 μM; see also Table VI) (50). Acyclovir and ganciclovir did not inhibit OAT4-mediated ES uptake, indicating that the spectrum of antivirals interacting with OAT4 is limited, at least from the extracellular side of this asymmetric transporter.

(c) Diuretics. OAT4 translocated radiolabeled bumetanide, but this uptake was much slower than that by OAT3 (60). In the same study, furosemide uptake by OAT4 was not significant. In inhibition experiments, OAT4 showed very low (trichlormethiazide, IC_{50} 1.5 mM; chlorothiazide, 2.6 mM) or no measurable affinity (cyclothiazide, hydrochlorothiazide) for thiazide diuretics, low (acetazolamide, 425 μM) or no affinity (methazolamide) for carbonic anhydrase inhibitors, and variable affinities for loop diuretics (bumetanide, 348 μM; ethacrynate, 8.76 μM; furosemide, 44.5 μM) (60). If, as our data suggest (112), OAT4 is an asymmetric carrier, these IC_{50} values determined for the interaction with the outside (luminal) binding site do not necessarily reflect the affinities with which OAT4 accepts diuretics from the cytosolic side.

(d) NSAIDs. Radiolabeled ketoprofen and salicylate were taken up by OAT4-expressing tubule cells, but this uptake, though statistically significant, was less than two times over mock (62). Acetylsalicylate, indomethacin, and ibuprofen did not show uptake above mock. In inhibition studies, the hydrophilic NSAIDs acetaminophen, acetylsalicylate and salicylate as well as phenacetin (no negative charge) exhibited no measurable affinity for OAT4. Medium affinities were observed for diclofenac (IC_{50} 34.5 μM), ibuprofen (103 μM), indomethacin (10.1 μM), ketoprofen (70.3 μM), mefenamate (61.7 μM), naproxen (85.4 μM), and

Table IV. Examples of Drugs Interacting with Human OAT4

Class	Tested Compounds	Reference
Antibiotics	benzylpenicillin, cefadroxil, cefamandol, cefazolin, cefoperazone, cefotaxim, ceftriaxon, cephaloridine, cephalotin, tetracycline[b]	(48,101,105,113)
Antiviral	zidovudine[b]	(50)
Antihypertensive	captopril	(102)
Cytostatic	methotrexate[b]	(113)
Diuretics	acetazolamide, bumetanide[b], chlorothiazide, ethacrynate, furosemide, trichlormethiazide	(60,101)
NSAIDs[a]	diclofenac, diflusinal, ibuprofen, indomethacin, ketoprofen[b], mefenamate, naproxen, phenylbutazone, piroxicam, salicylate[b], sulindac	(62,101,102,105,113)
Statin	pravastatin	(67,139)
Uricosuric	probenecid	(68,73,101,105,113,139)

[a] Abbreviations: *NSAID* non-steroidal anti-inflammatory drug
[b] Transport has been demonstrated

piroxicam (84.9 μM); sulindac showed a low affinity (617 μM) (62). On average, the affinities of hOAT4 for NSAIDs tend to be lower than those of hOAT1 and hOAT3, and higher than those of hOAT2 (see also Table VI). The low or absent transport suggests that OAT4 is not involved in absorption of NSAIDs from the tubule lumen. Since NSAIDs show a very low renal clearance, OAT4 may also not be involved in the release of these compounds.

(e) Uricosuric. Probenecid has been used as an inhibitor of OAT4. The K_i values determined are in the range between 44.4 and 67.7 μM (68,73,105). This figure is higher than the IC_{50} or K_i values for OAT1 (range 4.3–12.5 μM) and OAT3 (range 1.3–44 μM), but clearly smaller than that for OAT2 (766–977 μM). Thus, OAT4 has an intermediate affinity for probenecid that may, however, be sufficient to decrease urate absorption by this transporter.

OAT5–OAT9

A human, non-functional clone was isolated and named OAT5 (71), that is not related to the functionally characterized OAT5 from rat (109) and mouse (114). OAT6 to OAT9 have also been cloned (5), but a full description of their functional characteristics is not available at the time of writing this review.

URAT1

The urate transporter 1 was identified by screening the human genome data base for OAT-related genes (115). The gene (SLC22A12) is located on chromosome 11q13.1 and paired with the gene for OAT4 (28). The message is expressed in fetal and adult kidneys (115). The URAT1 protein is made of 553 amino acids and shows the typical predicted structure of 12 transmembrane helices. It is likely that the mouse homologue is the renal specific transporter, RST, that was cloned in 1997, but not functionally characterized (116).

Using antibodies, human and mouse URAT1 were localized at the apical membrane of renal proximal tubule cells (115,117). The apical location is most probably maintained by the interaction of URAT1 with the scaffolding protein PDZK1 (118). URAT1 binds through its three C-terminal amino acids threonine–glutamine–phenylalanine to the PDZ domains 1, 2 and 4 of PDZK1, and this binding increases V_{max} of transport at unaltered affinity for urate.

Endogenous substrates. The most important substrate of URAT1 is the anion of uric acid, urate. In humans, urate is the final product of the degradation of purine bases, whereas in rodents, urate is further metabolized to allantoin by the enzyme uricase (119). Due to the genetic loss of uricase, and the presence of the urate-absorbing URAT1 in the kidneys, plasma urate levels in humans are considerably higher (240–300 μM) than in rodents (30–120 μM). hURAT1 expressed in oocytes and HEK cells transported labelled urate with K_m values of 371 and 199 μM, respectively (115,118). Assuming that urate concentration in the glomerular filtrate is close to the above-mentioned plasma concentrations, URAT1 appears to be approximately half-saturated under physiological conditions. Urate uptake through URAT1 is driven by the efflux of lactate and nicotinate (115). Lactate is taken up across the apical membrane by the sodium monocarboxylate cotransporter, SMCT (SLC5A8; (120)). *In vitro*, urate/chloride exchange has also been observed (115), but given the physiological chloride gradient (lumen > cell) it is unlikely that urate is absorbed in exchange for chloride *in vivo*.

Mutations in the URAT1 gene are the cause of the idiopathic renal hypourecemia. Enomoto et al. were the first to report a missense mutation which leads to the occurrence of a premature stop codon (W258X) (115). Patients carrying this mutation had a low plasma urate level and a fractional renal urate excretion exceeding by far the ~10% observed in healthy subjects. Meanwhile a considerable number of mutations of URAT1 have been found to be associated with idiopathic renal hypouricemia (see below). These findings support the notion that URAT1 is the dominant transporter for urate uptake in renal proximal tubules.

Acetoacetate, ß-hydroxybutyrate, succinate, and α-ketoglutarate interacted with hURAT1, but a significant inhibition of urate uptake occurred only at high concentrations (10 mM) (115). Estrone sulfate, which is a substrate of many OATs, did not interact with human and mouse URAT1 (115).

Exogenous compounds. As shown in Table V, not so many drugs have been tested with hURAT1. The diuretics bumetanide and furosemide, and the NSAIDs indomethacin, phenylbutazone, salicylate and sulfinpyrazone inhibited hURAT-mediated urate transport (115). IC_{50} values are not known, and it is unclear whether hURAT1 translocates these substances. The angiotensin II receptor blocker losartan inhibited urate transport by human URAT1, which fits nicely to the observed uricosuric side effect of this drug. Benzbromarone and probenecid inhibited URAT1 (110,115,117), which again is in accordance with the uricosuric effect of these drugs. If the xanthine oxidase inhibitor allopurinol is administered for treatment of hyperuricemia and gout, its

Table V. Examples of Drugs Interacting with Human URAT1

Class	Tested Compounds	Reference
Antibiotic	benzylpenicillin[b]	(124)
Antihypertensive	losartan	(115)
Diuretics	bumetanide, furosemide	(115)
NSAIDs[a]	indomethacin, phenylbutazone, salicylate, sulfinpyrazone	(115)
Uricosurics	benzbromarone, probenecid	(110,115,117)

[a] Abbreviation: *NSAID* non-steroidal anti-inflammatory drug
[b] Transport has been demonstrated

metabolite oxypurinol itself acts as an inhibitor of URAT1, decreasing urate absorption and increasing the urate-lowering effect of allopurinol (110).

COMPARISON OF DRUG INTERACTIONS WITH OATS

Arrangement of OATs and driving systems. Figure 2 shows the arrangement in the proximal tubule cells of a human kidney of those OATs that have been discussed in this review. In the basolateral membrane, the Na^+,K^+-ATPase pumps three Na^+ ions out that return together with one α-ketoglutarate into the cell via the sodium-dicarboxylate cotransporter 3. α-Ketoglutarate is then exchanged via OAT1 or OAT3 against an organic anion delivered to the cell by the blood. Thus, Na^+,K^+-ATPase and NaDC3 are the "drivers," and OAT1 and OAT3 are being driven by the intracellular > extracellular α-ketoglutarate gradient. The driving force for OAT2 is not yet clear. At the apical membrane, the sodium lactate cotransporter pumps lactate, and NaDC1 dicarboxylates, from the filtrate into the cell. The intracellular > extracellular mono- and dicarboxylate gradients drive URAT1 and OAT4. The latter transporter can also be driven by the extracellular > intracellular Cl^- gradient, supporting organic anion efflux driven by chloride influx (112).

It has been proposed that the transporters in the apical membrane form functionally and—through scaffolding proteins—molecularly coupled units, the urate multimolecular complex, URAT-MMC, and the organic anion transporting complex, OAT-MMC (13). Components of the URAT-MMC would be URAT1 and SMCT for urate uptake (absorption) and NPT1 and MRP4 for urate release (secretion). The OAT-MMC would contain NaDC1 and OAT4 for organic anion uptake, and NPT1, MRP2 and MRP4 for organic anion release. Furthermore it has been proposed that the organic anion transporters at the basolateral cell side, OAT1 and OAT3, are driven by α-ketoglutarate, whereas those at the apical membrane, URAT1 and OAT4, interact with both, C4 dicarboxylates such as succinate and C5 dicarboxylates such as α-ketoglutarate (13). In this respect it is interesting that the basolateral sodium dicarboxylate cotransporter, hNaDC3, prefers α-ketoglutarate over succinate (121), whereas the apical transporter, hNaDC1, largely prefers C4 dicarboxylates (122).

Antibiotics. Benzylpenicillin interacted with human OAT1 (123), OAT3 (22,58), and OAT4 (101). Human OAT2 and URAT1 were not tested, but rOAT2 (77) and mURAT1/RST (124) were inhibited by benzylpenicillin. It is, therefore, likely that human OAT1-4 and URAT1 interact with this ß-lactam antibiotic. However, transport of benzylpenicillin has only been demonstrated for hOAT3, and a K_m (52.1 μM) was reported (22). For hOAT1, no K_m or IC_{50} values are available. For rOAT1, IC_{50} values range between 418 and 2.8 mM (44,88,97,125), and for rOAT3 between 52.8 and 132 μM (88,97,125), indicating that OAT3 has a clearly higher affinity for benzylpenicillin than OAT1. Therefore, rat and, most probably, human OAT3 may be the main players in renal benzylpenicillin excretion, and the target for the interaction with probenecid, which was originally developed to decrease renal loss of this antibiotic (126).

Cephalosporines were tested under the same experimental condition on human OAT1, OAT2, OAT3 and OAT4, and K_i values were obtained (48,79) and later corrected (127) (see respective chapters above and Table VI). hOAT1 showed decreasing affinities in the order cefamandole (lowest IC_{50}) > cefazolin, cefoperazone, cephalotin, ceftriaxon > cephaloridine > cephradine > cefotaxim > cefadroxil (highest IC_{50}). The respective sequence for hOAT2 was cefamandole > cephalotin, cefoperazone > cephaloridine > cefazolin, cefotaxim > cefadroxil, ceftriaxon; for hOAT3 cephalotin, cefamandole > cefotaxim > cefazolin, cephalexin > cefoperazone > cephaloridine > ceftriaxon > cefadroxil; and for hOAT4 cephalotin > cefamandole > cefazolin > cetriaxon > cefoperazone > cephaloridine > cefotaxim. Thus, OATs 1–4 have the highest or second highest affinity for cefamandol, and the lowest for cefadroxil (OAT4 was not tested). In between these two compounds, the sequences are similar, but not identical, indicating subtle differences in the structural requirements of OATs for cephalosporines. Taking the absolute K_i values (in mM), the affinities for cefamandole decreased in the order OAT1 (0.03) > OAT3 (0.046) > OAT4 (1.14) > OAT2 (1.57); and for the nephrotoxic cephaloridine in the order OAT1 (0.47) > OAT3 (2.46) > OAT4 (3.63) > OAT2 (4.48) (48; 79) (Table VI). Among the basolateral transporters, hOAT1 has the highest affinity for most cephalosporines, followed by OAT3, OAT4, and OAT2. If the V_{max} would be the same for all OATs, OAT1 would be the main player in the uptake of cephalosporines from the blood, and OAT2 would be of little importance. Since the V_{max} values of cephalosporine transport are not known, especially not in the intact kidney, this conclusion must remain preliminary. The OAT4, located at the apical membrane, showed for most cephalosporines a lower affinity than OAT1 and OAT3, and it has been speculated that OAT4 may be rate-limiting. Two caveats must be mentioned. First, OAT4 appears to be asymmetric, and we do not know the affinities for cephalosporines from the cytosolic side. Second, the transport rate of OAT4 relative to the rates of OAT1 and OAT3 is unknown. Hence, it is too early to conclude that OAT4 alone is responsible for the intracellular accumulation of cephalosporines and their nephrotoxic effects (128,129).

Comparative investigations have also been performed with the interaction of human OATs 1–4 with tetracyclines (47). All four OATs transported labelled tetracycline, although uptake was at best two times over mock. The K_m values for hOAT2, hOAT3, and hOAT4 were 440 μM, 566 μM, and 123 μM, respectively. K_m or IC_{50} values are not available for hOAT1, and for other tetracyclines (doxicycline, minocycline and oxytetracycline).

Figure 3 summarizes the available results for renal proximal tubular secretion of antibiotics. Again, we emphasize that V_{max} values for antibiotics are lacking in the intact tubule and, hence, Fig. 3 is speculative.

Antiviral drugs. Since antivirals can be nephrotoxic, there was a great interest in defining the transporters by which these compounds are taken up into proximal tubule cells. All tested drugs including acyclovir, adefovir, cidofovir, ganciclovir, tenofovir, zalcitabine, and zidovudine were transported by hOAT1 (see Table I for references). Human OAT2 transported zidovudine, but not acylovir, valacyclovir, and

Table VI. Drug Interaction with Human OATs: Overview of Reported $IC_{50}/K_i/K_m$ Values

	OAT1	OAT2	OAT3	OAT4
Antibiotics				
Benzylpenicillin	n. i.[b]/inhib.[c,d]		52.1[a]	inhib.[c]
Cefadroxil	6,140	6,400	8,620	
Cefamandole	30	430	46	1,140
Cefazolin	180	5,100	550	1,740
Cefoperazone	210	1,100	1,890	2,800
Cefotaxime	3,130	5,200	290	6,150
Ceftriaxone	230	6,800	4,390	2,380
Cephalexine			630	
Cephaloridine	740; 1,250[e]	2,100	2,460	3,630
Cephalotin	220	1,000	40	200
Cephradine	1,600			
Antivirals				
Acyclovir	342[a]	n. i.[b]; n. t.[g]	n. t.[g]	n. i.[b]; n. t.[g]
Adefovir	17.2–30[a,f]		n. t.[g]	
Cidofovir	46–58[a,f]			
Ganciclovir	896[a]	inhib.[c]; n. t.[g]	n. t.[g]	n. i.[b]
Tenofovir	22.3[a]			
Valacyclovir	n. t.[g]	n. t.[g]	transp.[h]	n. t.[g]
Zidovudine (AZT)	45.9[a]	26.8[a]	145.1[a]	151.8[a]
Diuretics				
Acetazolamide	75	>5,000	816	425
Bumetanide	7.6	n. t.[g]; 7.52[a,d]; 77.5	1.59[a]; 0.75	348
Chlorothiazide	3.78	2,205	65.3	2,632
Cyclothiazide	84.3	39.2	27.9	>5.000
Ethacrynate	29.6	121	0.58	8.76
Furosemide	18	603	7.31	44.5
Hydrochlorothiazide	67.3	1,023	942	>5,000
Methazolamide	438	>5,000	97.5	>5,000
Trichlormethiazide	19.2	1,220	71.2	1,505
NSAIDs				
Acetaminophen	639	>2,000	>2,000	>2,000
Acetylsalicylate	769	>2,000	717	>2,000
Diclofenac	4; 4.6[d]	14.3	7.78	34.5
Diflusinal	0.85			inhib.[c]
Etodolac	50			
Flurbiprofen	1.5			
Ibuprofen	8; 55.5[e]	692	6	103
Indomethacin	3; 3.83[e]	64.1	0.61	10.1
Ketoprofen	1.3; 1.4; 4.43[e]	400	5.98	70.3
Loxoprofen	27.1		8.7	
mefenamate	0.83	21.7	0.78	61.7
Naproxen	5.67; 5.8[e]	486	4.67	85.4
Phenacetin	200; 275[e]	1,878	19.4	>2,000
Piroxicam	19.8; 20.5; 62.8[e]	70.3	2.52; 4.88[e]	84.9
Salicylate	280; 325; 1,573[e]	>2,000	50	>2,000
Sulindac	36.2	440	3.62	617

[a] km; [b] no inhibition; [c] inhibition was observed, but IC_{50} or K_i was not reported; [d] conflicting results; [e] single values from different publications; [f] range of values from different publications; [g] not transported; [h] transport has been shown, but K_m was not determined. For literature see text.

ganciclovir; hOAT3 transported valacyclovir and zidovudine, but not acyclovir and ganciclovir; and hOAT4 translocated zidovudine, but not acyclovir, ganciclovir and valacyclovir (50). Thus, only zidovudine (AZT) is translocated by all four OATs. The K_m values were 45.9 μM for hOAT1, 26.8 μM for hOAT2, 145.1 μM for hOAT3, and 151.8 μM for hOAT4 (50) (Table VI). Taken together we assume that, in the basolateral membrane, hOAT1 transports all antiviral drugs, whereas hOAT2 and hOAT3 transport zidovudine (and OAT3 in addition valacyclovir). In the apical membrane, OAT4 appears to translocate only zidovudine, but did not interact with extracellular acyclovir and ganciclovir. Thus, whereas OAT4 may absorb zidovudine and thereby increase intracellular accumulation and cell toxicity, it remains to be clarified whether this asymmetric transporter is involved in the release of antiviral drugs. Possible additional transporters for antiviral release are the ATP-driven multidrug resistance transporters MRP2 and MRP4 (130,131). For a summary of the results see Fig. 4.

The expression of hOAT1 was shown to confer antiviral-mediated cytotoxicity (12,51), and the addition of NSAIDs to the medium attenuated this effect (54). Thus, NSAIDs could

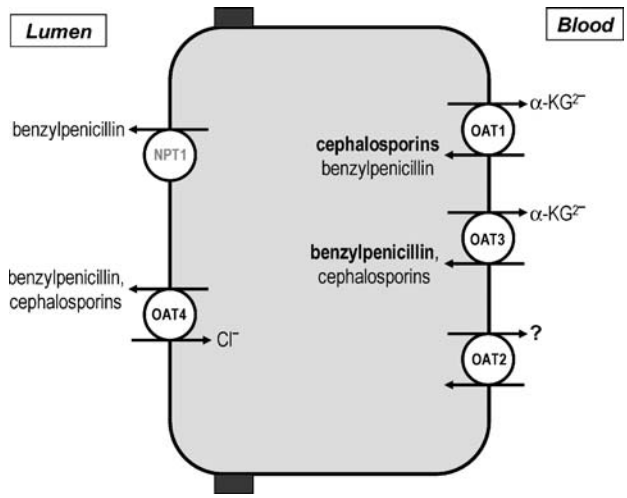

Fig. 3. Transport systems involved in human proximal tubular secretion of ß-lactam antibiotics. Based on the affinities, OAT1 in the basolateral membrane interacts mainly with cephalosporins, and OAT3 with benzylpenicillin. OAT2 has comparably low affinities for cephalosporins and probably does not contribute to secretion. In the apical membrane, OAT4 releases the antibiotics, perhaps in exchange for chloride ions. In addition, NPT1 alias NaPi-1 alias OAT_v1 has been described to transport benzylpenicillin (186,187). NPT1 does not belong to the OAT family. *Bold letters*, relatively high affinity; *normal letters*, relatively low affinity.

be used to decrease or prevent renal damage during antiviral therapy, provided their free plasma concentration is high enough to block OAT1. Probenecid is an excellent inhibitor of OAT1 and shows a relatively high free plasma concentration (about 20 μM (113)). Indeed, probenecid decreased renal clearance of antiviral drugs and is in use for prevention of nephrotoxicity (132–135).

Diuretics. The loop and thiazide diuretics act from the lumen side to inhibit the $Na^+,K^+,2Cl^-$-cotransporter NKCC2 in the thick ascending limb of Henle's loop, and the Na^+,Cl^--cotransporter NCC in the distal convoluted tubule, respectively. Since glomerular filtration is limited due to high plasma protein binding, proximal tubular secretion is the main route by which most diuretics gain access to the nephron lumen and, hence, to their target transporters. There is one comparative study on hOAT1–hOAT4 available which allows to compare the affinities ((60); see also Table VI). The affinity of hOAT1 decreased in the order chlorothiazide > bumetanide > furosemide, trichlormethiazide > ethacrynate > hydrochlorothiazide > cyclothiazide. The respective sequences for hOAT2 was cyclothiazide > bumetanide > ethacrynate > furosemide > hydrochlorothiazide > trichlormethiazide > chlorothiazide; for hOAT3: ethacrynate, bumetanide > furosemide > cyclothiazide > chlorothiazide, trichlormethiazide > hydrochlorothiazide; and for hOAT4: ethacrynate > furosemide > bumtanide ≫ trichlorometiazide > chlorothiazide ≫ cyclothiazide, hydrochlorothiazide (no measurable affinity). These sequences are considerably different, indicating that the OATs differ markedly with respect to their structural requirements for the interaction with diuretics. Among the loop diuretics, bumetanide interacted preferably with hOAT3 (IC_{50} 0.75 μM), followed by hOAT1 (7.6 μM), hOAT2 (77.5 μM) and hOAT4 (348 μM). Ethacrynate again interacted best with hOAT3 (0.58 μM), less well with hOAT4 (8.76 μM), hOAT1 (29.6 μM), and hOAT2 (121 μM). Furosemide exhibited another sequence: hOAT3 (7.31 μM), hOAT1 (18 μM), hOAT4 (44.5 μM) and hOAT2 (603 μM). In general, OAT3 has the highest affinity for loop diuretics, followed by OAT1 (bumetanide, furosemide) or OAT4 (ethacrynate). For thiazide diuretics, chlorothiazide (IC_{50} 3.78 μM), hydrochlorothiazide (67.3 μM), and trichlormethiazide (19.2 μM) had the highest affinity for hOAT1, and cyclothiazide for hOAT3 (27.9 μM). The lowest (trichlormethiazide, chlorothiazide) or a non-measurable affinity (cyclothiazide, hydrochlorothiazide) was found for the interaction with OAT4 (60). We obtained, however, experimental evidence for an interaction of OAT4 from the cytosolic side with hydrochlorothiazide (Hagos et al., unpublished), suggesting that OAT4 may well be involved in the release (secretion) of thiazides, whereas it has no affinity for the uptake (absorption) of thiazides.

Based on the IC_{50} values, it appears that at the basolateral membrane OAT1 acts as the preferred entry for thiazides (exception: cyclothiazide), and OAT3 for loop diuretics, respectively. OAT2 may contribute to the uptake of cyclothiazide (see Fig. 5). This assumption is only valid, if OAT1, OAT2 and OAT3 have similar V_{max} values for the diuretics. These values are, however, not yet available, leaving a final conclusion open. The exit of diuretics across the apical membrane could be accomplished by OAT4 (for a summary see Fig. 5).

Non-steroidal anti-inflammatory drugs (NSAIDs). Human OAT1, OAT2, OAT3, and OAT4 were shown to interact with NSAIDs (see Tables II,III,IV and V for references). In addition, all four OATs have been tested under the same

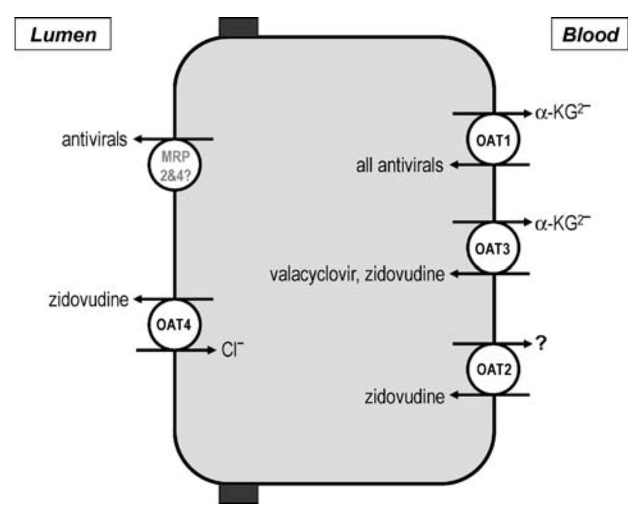

Fig. 4. Transport systems involved in human renal proximal tubular secretion of antiviral drugs. At the basolateral membrane, hOAT1 transports probably all antiviral drugs, whereas OAT2 and OAT3 interact with one or two selected compounds. At the apical membrane, OAT4 may transport zidovudine (*AZT*). Whether other antivirals are transported in the secretory direction by the asymmetric OAT4, remains to be determined. MRP2 and MRP4 may transport some antivirals out of the cell, but it needs to be clarified whether other transporters are also involved. The multidrug resistance related proteins (*MRPs*) do not belong to the family of OATs.

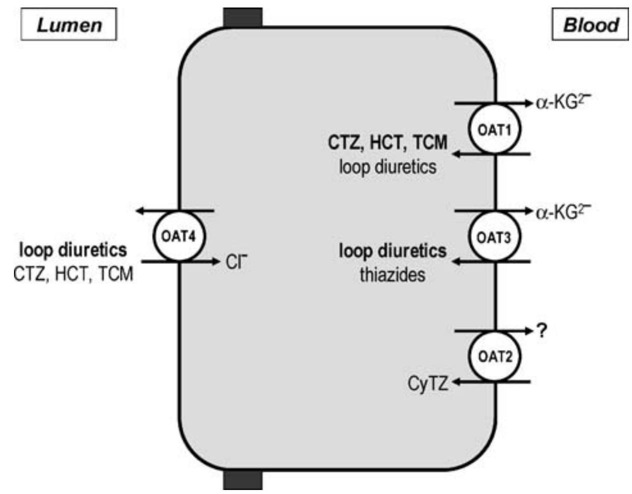

Fig. 5. Proximal tubular secretion of diuretics. At the basolateral membrane, OAT1 has the highest affinities (*bold letters*) for chlorothiazide (*CTZ*), hydrochlorothiazide (*HCT*) and trichlormethiazide (*TCM*), and generally less affinity (*normal letters*) for loop diuretics (bumetanide, ethacrynate, furosemide). OAT3 prefers loop diuretics over thiazides, and OAT2 has a high affinity for cyclothiazide (*CyTZ*). At the apical membrane OAT4 may release most diuretics, although it has a low affinity particularly for thiazides.

experimental condition, allowing for a comparison of IC_{50} values ((62); Table VI). The hydrophilic NSAIDs acetaminophen, acetylsalicylate and salicylate interacted with hOAT1 (IC_{50} values 638, 769, and 325 μM, respectively), and two of them with OAT3 (acetylsalicylate, IC_{50} 717 μM; salicylate, 50 μM). No inhibition was found for hOAT2 and OAT4. The hydrophobic NSAIDs generally interacted with much lower IC_{50} values at hOAT1, ranging between 0.83 μM for mefenamate and 175 μM for phenacetin, and at hOAT3 (between 0.61 μM for indomethacin and 19.4 μM for phenacetin). On average, hOAT2 and OAT4 were less sensitive than OAT1 and OAT3 for hydrophobic NSAIDs (IC_{50} at OAT2 between 14.3 μM and 1.88 mM; and at OAT4 between 10.1 μM and >2 mM).

An interaction between NSAIDs and methotrexate has been implicated as a cause of severe side effects (see literature in (113)). Methotrexate is transported by human OAT1 (K_m 724 μM (63)), OAT2 (no K_m available (71)), OAT3 (K_m 10.9 μM (58)), and OAT4 (K_m 17.8 μM (113)). Since all OATs are inhibited by NSAIDs, drug–drug interaction could occur at any of these transporters. If the free plasma concentrations are taken into account, salicylate (K_i at hOAT3 1,020 μM, free conc. 431 μM); phenylbutazone (34.7/12.5 μM); indomethacin (6.0/8.4 μM); and loxoprofen (8.7/20 μM) could substantially inhibit OAT3-mediated methotrexate transport and, hence, uptake from the blood into proximal tubule cells. Salicylate (IC_{50} values at OAT1 between 280 and 1.57 mM (42,49,62)), phenylbutazone (IC_{50} 47.9 μM (136)), indomethacin (IC_{50} between 3.0 and 3.8 μM (54,62)) and loxoprofen (IC_{50} 27.1 μM (63)) should also substantially inhibit hOAT1, decreasing cellular uptake of methotrexate further. The IC_{50} values for hOAT2 (salicylate, >2 mM; indomethacin, 64.1 μM (62)) and OAT4 (salicylate, >2 mM; indomethacin, 10.1 μM (62)) suggest that only indomethacin could substantially inhibit OAT4 *in vivo*. Taken together, salicylate, phenylbutazone, indomethacin, and loxoprofen could be responsible for methotrexate–NSAID interaction at OAT1 and OAT3, and indomethacin also at OAT4 (63,113,136).

The same NSAIDs potentially involved in interaction with methotrexate could be used to prevent nephrotoxicity by antivirals (54) and other potentially nephrotoxic substances that are transported by OATs. As regards transport, salicylate was transported by all OATs, indomethacin by OAT1 and OAT3, ketoprofen by OAT1, OAT3, and OAT4, and ibuprofen by OAT1 and OAT3 (62). In all cases, transport was at best two times greater than uptake into mock. Thus it seems that NSAIDs do interact with OATs, and can inhibit them, but are not effectively translocated. This explains the very low renal excretion which for many NSAIDs amounts to less than 1% of total clearance. Higher numbers were found only for salicylate (2–30%), indomethacin (16%), and piroxicam (4–10%) (62).

INVOLVEMENT OF OATS IN TOXICITY

Nephrotoxic drugs. This topic was elegantly reviewed by D. H. Sweet (12). Here, we only briefly touch this topic. It is highly likely that OAT1 and OAT3 contribute significantly to nephrotoxicity by taking up cephaloridine and antiviral drugs from the blood into the cells. Particularly, proximal tubule cells are exposed to cytotoxic drugs because they express OAT1 and OAT3. ß-Lactam antibiotics are in addition taken up from the apical cell side by the H^+-peptide cotransporter PEPT2 (137) and thus a high intracellular concentration can be achieved. Strategies to prevent the nephrotoxicity of antibiotics involve the use of inhibitors of OATs such as cilastatin and betamipron (66,129). The use of NSAIDs (54) and probenecid (132–134) can reduce renal excretion of antiviral drugs and of nephrotoxity. Thereby, intentional drug–drug interaction provides a means to prevent organ damage. As pointed out in (12), inhibition of OATs has not only an impact on the kidneys, but also on liver and brain. Inhibition of OATs in the liver could impair drug metabolism, and inhibition of OATs in choroid plexus and the blood brain barrier could prevent the removal of drugs from the brain and cause cerebral symptoms.

Uremic toxins. During renal failure, several organic anions are accumulated in the plasma and cause side effects. These compounds called uremic toxins include indoxyl sulfate (IS), indole acetate (IA), hippurate (HA), and 3-carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF) (138). IS and CMPF themselves cause renal failure and aggravate the situation. HA and CMPF are made responsible for neurological disorders accompanying the renal failure, HA interferes with the glucose utilization in skeletal muscle, and CMPF inhibits the binding of drugs to albumin (138). All these uremic toxins are substrates of hOAT1. Indoxyl sulfate was transported with a K_m of 20.5 μM, and inhibited OAT1-mediated transport with IC_{50} values between 13.2 and 83 μM (125,139,140). Indole acetate was taken up by hOAT1 with a K_m of 14 μM, and inhibited transport with IC_{50} values 21 and 83 μM (125,140). Hippurate uptake occurred with a K_m of 23.5 μM, and inhibition of hOAT1 with an IC_{50} of 18.8 μM;

the respective numbers for CMPF are 141 μM for K_m and 247 μM for IC_{50} (125). IS and IA uptake increased the production of oxygen radicals, and probenecid inhibited this effect (141). Indoxyl sulfate interacted also with OAT3 (K_m 263 μM; K_i 169 μM (125,139)) and OAT4 (K_i 181 μM (139)), indole acetate (no transport, but inhibition with IC_{50} of 491 μM), hippurate (no transport, but inhibition with IC_{50} of 30.8 μM), and CMPF with OAT3 (K_m for uptake 26.5 μM; IC_{50} 27.9 μM) (125). Thus, OAT1 and OAT3 are involved in the uptake of uremic toxins and their excretion. In renal failure, however, the capacity to excrete uremic toxins is decreased. Piling up the toxins inhibits the remaining OAT1 and OAT3 progressively, which could cause severe problems with the excretion of antibiotics, methotrexate, antivirals, etc.

Environmental toxins. Again we refer to (12) for an in-depth discussion of this issue. The herbicide 2,4-dichlorophenoxyacetate (2,4-D) is a substrate of hOAT1 ((26, 52); K_m for uptake 5.77 μM (22)) and hOAT3 (weak transport (26)). *N*-acetyl-L-cysteine *S*-conjugates resulting from glutathione conjugation of toxic compounds are substrates of rOAT1 (142) and probably also of human OAT1. The cysteine conjugates *S*-benzothiazolcysteine, *S*-chlorotrifluoroethylcysteine, and *S*-dichlorovinylcysteine inhibited hOAT1 with IC_{50} values of 9.9, 177, and 208 μM, respectively (143). Complexes between mercury and *N*-acetyl-L-cysteine (NAC–Hg, K_m 44 μM (144); NAC–Hg–CH_3 (145), K_m 79.5 μM; NAC–Hg–NAC, K_m 144 μM (146)), L-cysteine (Cys–Hg (144); Cys–Hg–Cys, K_m 91 μM (147)), and homocysteine (Hcy) (CH_3–Hg–Hcy, K_m 39 μM (148); Hcy–Hg–Hcy (149)) are transported by hOAT1, and NAC–Hg by hOAT3 (26). Cell toxicity of Hcy–Hg complexes was higher in OAT1-expressing cells than in mock (149). These findings explain why mercury is accumulated particularly in proximal tubule cells (150). Fortunately, the same transporters, OAT1 (24,145,151) and OAT3 (152) can be used to direct an antidote, 2,3-dimercaptopropane-1-sulfonate (DMPS), into proximal tubule cells to chelate the mercury, facilitating greatly its excretion. Another heavy metal chelator, 2,3-dimercaptosuccinate (DMSA, succimer), is transported by NaDC3 (153) that is also located in the basolateral membrane (c.f. Fig. 2).

Carcinogenic compounds are also transported by OATs. A prominent example is ochratoxin A that is translocated by hOAT1 (K_m 0.42 μM (64)), hOAT3 (K_m 0.75 μM (64)), and hOAT4 (K_m 22.9 μM (105)). Recently, it has been shown that sulfoxymethyl pyrenes (SMP) are substrates of hOAT1 and hOAT3 (154). At hOAT1, 2- and 4-sulfomethoxypyrenes showed K_i values of 4.4 and 5.1 μM, respectively; at OAT3, the respective K_i values were 1.9 and 2.1 μM. The expression of OAT1 and OAT3 increased the number of SMP–DNA adducts, and probenecid completely prevented this effect. Thus it appears that at least OAT1 and OAT3 can be involved in renal carcinogenesis by taking up ochratoxin A and SMPs from the blood into proximal tubule cells.

FACTORS INFLUENCING THE ACTIVITY OF OATS

There is no doubt that the activity of OATs influences renal drug elimination and hence pharmacokinetics. Any decrease in OAT activity should increase the body's exposure to drugs and could cause unwanted side effects. Here we summarize shortly conditions that have been shown to have an impact on the expression and activity of renal OATs.

Gender differences. In rats, gender differences have been observed for OAT1, OAT2, and OAT3. The renal expression of mRNA for OAT1 rose after birth equally for male (M) and female (F) animals; after puberty, message for F decreased whereas that for M stayed constant (155). In accordance, the abundance of OAT1 in the S2 segment was greater in M than in F, and testosterone treatment upregulated, and estradiol treatment decreased the protein amount (36). In mice, similar gender differences were observed (156,157), but in rabbits, no difference was found between M and F animals (157). After birth, rat renal mRNA expression for OAT2 stayed low for M, but rose sharply after puberty for F (155). In mice, no difference in OAT2 mRNA was found (156). In immunohistological studies, OAT2 protein was clearly more expressed in F rats and mice than in M animals (75). For rat renal OAT3, no differences were observed in mRNA expression (155) and abundance of protein except for a small change in proximal tubular expression that was higher in M than in F (36). In mice, gender differences depended on the strain (156). In rabbits, neither OAT2 nor OAT3 exhibited differences between M and F animals (157). As opposed to kidneys, there is a clear difference in hepatic OAT3 expression: M rats showed more mRNA (155) and protein (Sabolic et al., unpublished) than F rats. Finally, it has been shown for mouse URAT1/RST that M animals express more of this urate transporter than F mice (117).

So far, it is not known whether gender differences exist in humans. It is, however, likely that URAT1 is present at higher levels in men, because men have higher blood urate levels than women (for discussion see (117)). An androgen-responsive element has been found in the URAT1 promoter (158), supporting the assumption of gender differences in URAT1 expression.

Single nucleotide polymorphisms (SNPs). SNPs in drug transporter genes may have a not yet fully appreciated impact on pharmacokinetics and the occurrence of side effects (14). With regard to OATs, there are several reports on the occurrence of SNPs both in coding and non-coding (promoter, introns) regions. Here we only deal with non-synonymous changes in coding regions. For hOAT1, the following amino acid exchanges were reported: L7P (159); R50H (53,159,160); P104L (160); F160L (161); I226T (160); A256V (160); P283L (161); R287G (161); A256W (160); P341L (161); R454Q (160); K525I (53). The SNP R50H (located in the large extracellular loop between TM1 and TM2) was observed in African-Americans and Mexican-Americans with allele frequencies of 0.032 and 0.01 (160). In another study, the SNPs R50H and K525I showed frequencies of 0.04 and 0.005, respectively (53). When introduced into OAT1 and expressed in *Xenopus laevis* oocytes, the mutant K454Q (located at the cytoplasmic beginning of TM11) was non-functional. All other mutants showed probenecid-inhibitable uptake of *p*-amino-hippurate, ochratoxin A and methotrexate (160). The K_m values for PAH (53,160) and ochratoxin A (160) were unchanged. When the K_m values for adefovir, cidofovir and tenofovir were determined, R50H, showed a significantly increased affinity towards these antiviral nucleoside phosphonates (53). Patients carrying the R50H mutation may be more susceptible to renal damage because of a more effective uptake of nephrotoxic antiviral drugs. Unexpectedly, two persons carrying the K545Q mutant leading to a non-functional OAT1 did not show a decreased adefovir clearance. Since these persons were heterozygous for this mutation, the remaining functional OAT1 may have been sufficient to transport adefovir, and/or other steps are rate-limiting in adefovir secretion (160).

For OAT3 the mutation I175V in a Japanese individual was reported (159). In a survey including 270 Americans of different descent, ten SNPs were found that led to mutations within OAT3 (162): F129L, R149S, N239Stop, I260R, R277W, V281A, I305F, A310V, A399S, and V448I. The V281A mutant showed an allele frequency of 6% in African-Americans, and I305F a frequency of 3.5% in Asian-Americans. After expressing all mutants in HEK293 cells, three mutants, R149S (location: intracellular loop between TM2 and TM3), N239X (TM6), and I277W (loop between TM6 and TM7), did not transport estrone sulfate (ES) and cimetidine (CIM) (162). Two mutants (R277W, I305F) showed reduced ES transport, but only R277W had also reduced CIM transport. I305F appeared to have an altered substrate specificity in transporting CIM better, and ES worse, than the wildtype OAT3. All other mutants exhibited an unaltered transport, at least with respect top ES and CIM (162).

For OAT2, three non-synonymous SNPs leading to the mutations T110I, V192I and G507D were reported with allele frequencies of around 1% in the overall samples (159). In the same study, eight non-synonymous changes were found for OAT4 with the amino acid substitutions V13M, R48Stop, T62R, V155M, A244V, E278K, V399M, and T392I. One Subsaharan person had two mutations (G37A; G463A). Functional investigations on these mutants are not available.

Synonymous SNPs in URAT1 are relatively frequent (159). Many non-synonymous changes occurring predominantly in Japan and Korea are associated with familial idiopathic hypourecemia. These changes include: R90H (163–166), V138M (166), G164S (166), T217M (115,166), W258X (115,163,165–168), E298D (115), Q382L (166), M430T (166), R477H (163). The most frequent mutation with 74.1% of all patients is W258X which leads to a truncation of the URAT1 (166). When the mutants R90H, V138M, G164S, Q382L, M430T (166), and W258X (115) were expressed in oocytes, no or strongly reduced transport of urate was observed. Non-functional or hypoactive URAT1 in the apical membrane then reduces urate reabsorption and, thereby, serum urate levels (hypouricemia).

Disease-related down-regulation of OATs. Some drugs and toxins were reported to down-regulate the expression of OATs in liver. The activation of “drug-sensing receptors” AhR, CAR, PXR, and Nrf2 by their respective ligands changed the expression of a number of transporters in the hepatocyte (169). Whereas MDR1, MRP2, MRP3, BCRP and OATP-C were upregulated, a decreased expression was found for MRP6, BSEP, OCT1, OATP-B, OATP8, NTCP, and OAT2. Particularly phenobarbital (acting through CAR) effectively decreased OAT2 expression, whereas the activation of other receptors had smaller effects (169). Hepatic mRNAs for OCT1 and OAT3, but not for OAT2, were decreased in rats treated with lipopolysaccharide (LPS) (170).

In the kidneys, both down- and up-regulation of OATs was observed under various conditions. A bilateral ureteral obstruction for 24 h decreased renal *p*-aminohippurate excretion, but increased the amount of OAT1 protein in Western blots (171). A biliary obstruction for 3 days (172) did not change the abundance of total OAT1 protein in rat kidneys, but decreased the amount of OAT1 located in the basolateral membrane. Possibly, OAT1 was partially cleared from the basolateral membrane by endocytosis, and protein kinase C activation may have played a role in this process. OAT3 abundance was increased both, in the total kidney and in the basolateral membrane (172). Chronic renal failure induced by 5/6 nephrectomy decreased OAT1, but not OAT3, in the basolateral membrane (173). Finally, prostaglandin E_2 dose- and time-dependently reduced mRNA and protein of OAT1 and OAT3 in rat kidneys (174). At an exposure time of 48 h, a half-maximal effect on the decrease of OAT1 and OAT3 protein was observed at 23 and 27 nM PGE_2, respectively. Thus, PGE_2 has two opposing functions: at short exposure times it increases (175–177), and at long times it decreases the function of OATs.

OUTLOOK

Experimental data suggests that OATs are involved in drug transport in kidneys (OAT1-4, URAT1), liver (OAT2, OAT3), and brain (OAT1-3). The interaction of expressed OATs with several classes of drugs is well-documented. Since OATs from human origin are available, drug transport specificities could have a direct bearing on drug delivery and excretion in man. There are, however, some fields that should be considered in future experiments. First, despite the many tested drugs and other experimental substrates, a pharmacophore model is missing for all OATs. Thus, a prediction of structural requirements for interaction with, and transport by, OATs is not yet possible. Second, in nearly all cases we cannot tell which of the transporters is the main player, and which is rate-limiting in, e.g., renal drug excretion. Measuring renal drug excretion in the presence of OAT-isoform specific inhibitors may help to answer this question. Third, it has been largely overlooked that protein binding of drugs has a large impact on the availability of these compounds for OATs. In a recent study, addition of albumin abolished the transport of ochratoxin A by OAT1 and OAT3 and reduced estrone sulfate uptake by OAT3 (178). IC_{50} or K_i values determined *in vitro* without albumin must be related to the free, not to the total plasma concentrations of the drugs in order to appreciate the potential importance for a given OAT in drug transport. Fourth, OATs may be the site not only of drug–drug interactions, but also of interactions of endogenous substrates (urate, prostaglandins, neurotransmitter metabolites) and of foodstuff (e.g. phenolic compounds; N. A. Wolff, unpublished; caffeine (45,179)) with drugs. Finally, the search for SNPs has just begun. A relation to disease is obvious for URAT1 and hypouricemia, but the impact of mutations of other OATs on pharmacokinetics and occurrence of side effects is as yet unclear. Taken together, OATs will remain a field of active research, and we hope that a deeper understanding of these poly-specific transporters may help towards a better therapy.

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