



Are mice, rats, and rabbits good models for physiological, pharmacological and toxicological studies in humans?

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Abbreviations:

BBM – brush-border membrane
BLM – basolateral membrane
PT – proximal tubules
OA – organic anions
OC – organic cations

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Abstract

In the mammalian kidneys, handling of various organic compounds is mediated by multispecific organic anion and cation transporters localized in the luminal and contraluminal cell membrane domains of specific nephron segments, largely in proximal tubules. These transporters are responsible for cellular uptake and/or elimination of endogenous and xenobiotic organic compounds, including various anionic and cationic drugs, thus contributing to their reabsorption and/or secretion along the nephron. Recent studies have indicated a pivotal role of these transporters in drug resistance, drug-drug interactions, and drug-induced nephrotoxicity, whereas the presence of dysfunctional transporters due to truncated isoforms or point mutations can cause genetic diseases. In rat, mouse and rabbit nephrons, a number of these transporters exhibit sex differences in their protein and/or mRNA expression. In comparison with the expression in rodents and rabbits, in the human nephrons some transporters are absent, some exhibit different localization in the cell membrane domains, and none exhibit the sex-dependent expression. Species differences in some transporters have been further demonstrated concerning substrate selectivity, distribution in cells along the nephron, levels of mRNA and/or protein expression, sensitivity to inhibitors, and regulation. Overall these differences in the mammalian kidneys indicate that: a) data on the membrane transporters-related functions in one species can not simply be regarded as relevant for other species, and b) many physiological, pharmacological, and toxicological findings related to organic anion and cation transport and transporters in rodents and rabbits do not reflect the situation in humans.

INTRODUCTION

Although the exact number of animals used annually for various experimental purposes worldwide is not known, it has been estimated that between 40 million and 100 million of various animals are used, most of them (~80%) being the purpose-bred rodents (mainly mice and rats, but also hamsters, guinea pigs and gerbils) (http://en.wikipedia.org/wiki/Animal_testing). These animals are used for educational purposes, in basic research (genetics, physiology, biomedicine, developmental biology, behavioral studies, basic pharmacology) and in applied research (surgery, drug development, testing of various toxic and cosmetic substances, military research). In pharmacological and toxicological studies, especially when testing drugs, mice and rats are the most common animals used. The 3R-alternatives (Reduction, Re-

finement, Replacement), including non-animal alternatives, have been widely accepted as the way to diminish the use of experimental animals in research and testing, but when studying interactions among cells, tissues and organs, or when testing pharmacology and toxicology of various substances, including drugs to be used in human and veterinary medicine, in most cases there is no plausible substitute for the living animal. Moreover, the current legislation requires that all new drugs, before being licensed for human and animal use, have to be rigorously tested in at least two mammalian species (rodents AND non-rodents) for metabolism, pharmacokinetics, acute and chronic toxicology in adult species, efficacy regarding the expected actions, effects on reproduction, embryonic toxicity, and potential carcinogenicity. However, the increasing evidence indicate that rodents and some other common experimental animals, such as rabbits, may not be good models for such studies relevant to humans due to sex and species differences in various properties. Particularly relevant to this problem are the data on the role and expression of various membrane transporters that mediate transport of organic anions (OA) and cations (OC) in the mammalian kidneys and other organs. In recent years, a number of these transporters from different protein families and species have been cloned and characterized, and their localization, mainly in the liver and kidneys, but also in the intestine, brain and placenta, have been studied. Their kinetic and functional characteristics, sex and species differences in their expression, and their relevance for drug transport, drug-drug interactions and drug toxicity, have been extensively studied in normal and specific gene-inactivated (knock-out) animals, as well as in humans, and discussed in numerous recent reviews (1–22).

ORGANIC ANION AND CATION TRANSPORTERS IN THE MAMMALIAN KIDNEY

Humans and animals are constantly exposed to various organic compounds that are either produced during normal metabolism (endogenous substances) or enter the body *via* food, air, or medication (exogenous compounds, xenobiotics), and are potentially harmful to their health. In the body, these compounds are either biotransformed into more or less active metabolites, largely in liver and kidneys, or remain unchanged. Dependent on their physico-chemical characteristics, at the physiological pH these organic compounds behave as OA (negatively-charged) or OC (positively-charged), whereas some compounds may be both (zwitter-ions). The unchanged or biotransformed organic compounds are eliminated from the body partially by the liver and largely by the kidneys *via* secretory processes that are mediated by various transporters localized in the cell membrane. The representative groups of endogenous and xenobiotic OA and OC, which are handled by the mammalian liver and kidneys, are listed in Table 1. The cell membrane is not freely permeable to these compounds; they are trans-

ported by various specialized proteins localized in the membrane, collectively named as »drug transporters«.

Detailed lists of these compounds associated with relevant transporters in the animal and human kidneys have been reviewed elsewhere (1, 2, 4, 5, 8, 10, 11, 13, 16, 17, 22).

In the mammalian kidneys, transport of OA and OC is maintained by numerous, largely multispecific transporters that are localized in the apical (luminal) and/or basolateral membrane domains in the epithelial cells along the nephron. To drive vectorial transport of their substrates in direction of secretion or reabsorption, most OA transporters operate as bidirectional anion exchangers, and use transmembrane ion gradients (secondary- or tertiary-active transporters) generated by the activity of primary-active Na/K-ATPase and/or secondary-active ion exchangers (for example, Na⁺/H⁺ antiporter), whereas most OC transporters operate as the bidirectional facilitators, while some OA and OC transporters use ATP (primary-active transporters). The characteristics and the nomenclature of all these transporters have been recently collected and published; most of them belong to the large family of solute carriers (Slc in animals/SLC in humans), whereas the ATP-driven transporters are mem-

TABLE 1

Representative endogenous and exogenous (xenobiotic) OA and OC that are handled by various membrane transporters in the mammalian kidneys.

Organic anions
Endogenous: cyclic nucleotides, dicarboxylates, urate, folate, some prostaglandins, neurotransmitter metabolites, steroid hormones conjugated with sulfate, cysteine, glycine, and glucuronide.
Exogenous (xenobiotics): Medicaments (antibiotics, anti-viral drugs, nonsteroid anti-inflammatory drugs, diuretics, angiotensin-converting enzyme inhibitors, angiotensin II antagonists, anti-neoplastics, anti-epileptics, histamine-H ₂ -receptor antagonists); Conjugates (steroid hormones conjugated with sulfate, cysteine, glycine, and glucuronide); Diagnostic and experimental drugs (p-aminhippuric acid (PAH)); Food constituents (flavonoids); Environmental toxins (mycotoxins, herbicides, pesticides, some toxic metals).
Organic cations
Endogenous: choline, corticosterone, progesterone, endogenous cardiac glycosides, bioactive monoamines (dopamine, histamine, epinephrin, norepinephrin, serotonin), some prostaglandins.
Exogenous (xenobiotics): Medicaments ((ant)agonists of various receptors, ion channel blockers, transporter blockers, psychoactive drugs, some antiviral drugs, antidiabetics, anesthetics, antimalarics, muscle relaxants, cardiac glycosides); Toxins and experimental drugs (tetraethylammonium (TEA), tetramethylammonium (TMA), nicotine).
Zwitter ion – L-carnitine

Detailed lists of these compounds associated with relevant transporters in the animal and human kidneys have been reviewed elsewhere (1, 2, 4, 5, 8, 10, 11, 13, 16, 17, 22).

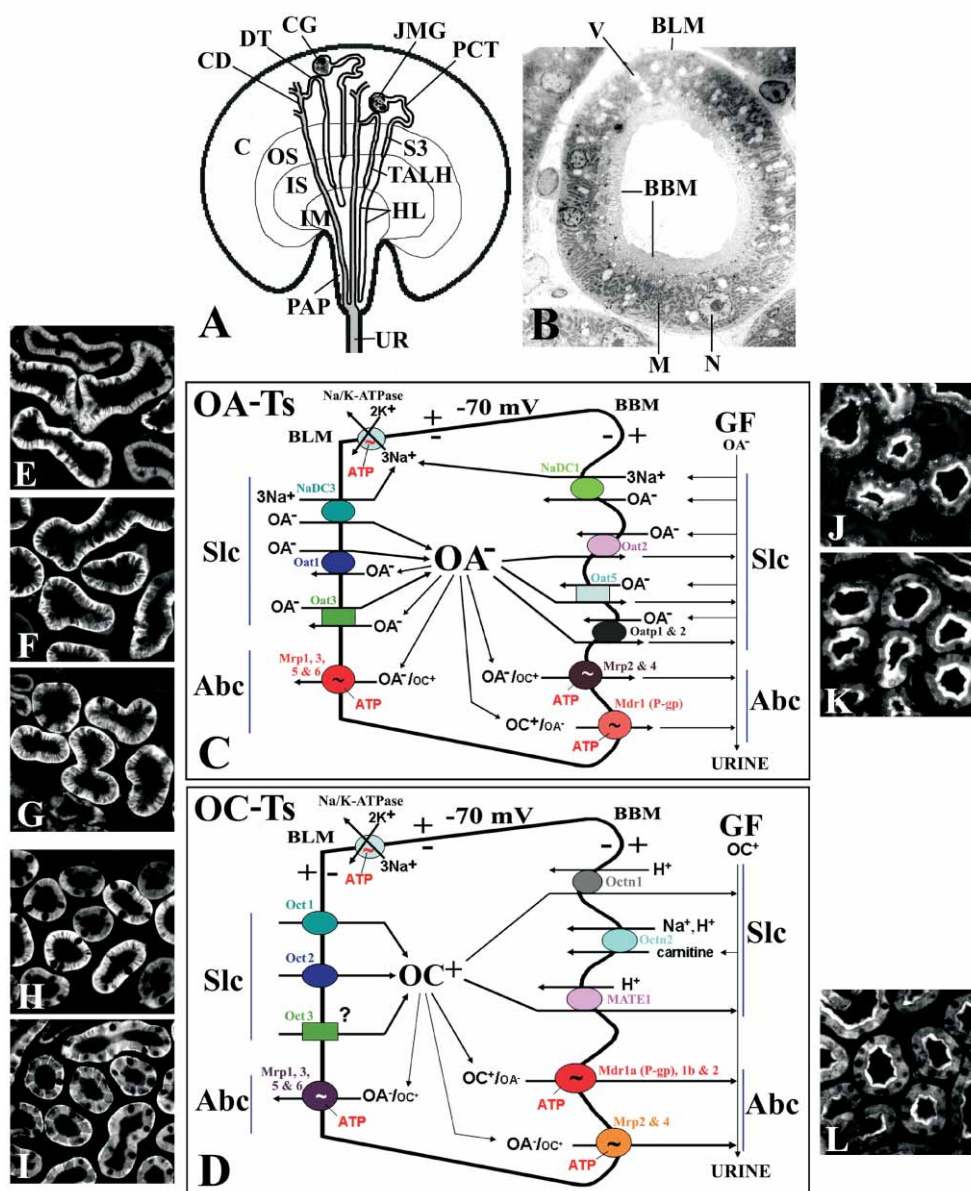


Figure 1. Polar distribution of the common drug transporters in the rat PT cells. **A.** Definition of various nephron segments in relation to specific tissue zones. C, cortex; OS, outer stripe; IS, inner stripe; IM, inner medulla; PAP, papilla; UR, ureter; CG, cortical glomerulus; JMG, juxtamedullary glomerulus; PCT, proximal convoluted tubule; S3, PT straight segment; DT, distal tubule; CD, collecting duct; TALH, thick ascending limb of Henle; HL, Henle loop. **B.** Transmission electron micrograph of a PCT (cross section). BLM, basolateral (contraluminal) membrane; V, vacuole; BBM, brush-border (luminal, apical) membrane; M, mitochondria; N, nucleus. **C.** Membrane domain-specific distribution of various OA transporters (OA-Ts) in the PT cell. Slc (solute carriers) are secondary- or tertiary-active transporters: sodium-dicarboxylate cotransporter NaDC3 and sodium-independent OA exchanger Oat1 and 3 are located in the BLM, whereas NaDC1, Oat2 and 5, and Oatp1 and 2 are located in the BBM. The driving force for all these transporters provides the ATP-driven (primary-active) Na/K-ATPase in the BLM, which generates ion gradients and the transmembrane (inside-negative) membrane potential. Abc (ATP-binding cassette) are all ATP-driven (primary-active) transporters, located in either BLM (multidrug resistance associated proteins Mrp1, 3, 5, and 6) or BBM (Mrp2 and 4), which predominantly accept OC as substrates, and several multidrug resistance proteins from the Mdr1 (P-gp) subfamily in BBM, which predominantly accept OA, but also some OC as substrates. At the BBM, some OA from the glomerular filtrate (GF) can be reabsorbed, whereas at the BLM, most OA (drugs) are transported and accumulated in the cell, and then secreted across the BBM into the tubule luminal fluid. **D.** Membrane domain-specific distribution of various OC transporters (OC-Ts) in the PT cell. Slc: The OC transporters Oct1-3 in the BLM operate as facilitators that transport OC into the cell using the inside-negative membrane potential (generated by the Na/K-ATPase and ion gradients) as a driving force. Thus accumulated OC can exit the cell partially via the Abc transporters (Mrp1, 3, 5, 6) in the BLM, and predominantly via the BBM transporters, e.g., H⁺/OC exchangers Ocn1 (organic cation novel membrane transporter) and MATE1 (multidrug and toxin extruder), Mdr1 (P-gp) proteins, and (less with) Mrp2 and 4. Some OC and the zwitterion L-carnitine can be reabsorbed from the glomerular filtrate by the action of Na⁺ (or H⁺)-OC cotransporter Ocn2. The non-reabsorbed and secreted OA or OC finish in urine. **E-L.** Representative pictures, obtained in immunocytochemical studies using specific antibodies (26–28, and our unpublished results), showing localization of a few OA and OC transporters in the PT BLM (E-I) and BBM (J-L). E, Na/K-ATPase; F, Oat1; G, Oat3; H, Oat1; I, Oat2; J, Oat2; K, Oat5; L, Mdr1.

bers of the ATP-binding cassette (Abc in animals/ABC in humans) family of transporters (23, 24). The number of newly discovered, cloned, and characterized transporters from both families increases every year. In the nephron, these transporters mediate: a) transport (net secretion) of various endogenous organic compounds that are generated in normal metabolism in the kidneys and other organs, b) transport (net secretion) of exogenous (xenobiotic) organic compounds that enter the body for iatrogenic (medicaments/drugs) or alimentary (food constituents) reasons, or as the environmental toxins, c) drug-drug interactions, d) drug resistance, e) development of drug-induced nephrotoxicity, and f) specific genetic diseases, caused by malfunctional transporters due to truncated isoforms or point mutations. The convoluted (S1/S2) and straight (S3) proximal tubule (PT) segments are the principal nephron parts in which handling of OA and OC takes place. In the PT epithelial cells, the relevant transporters are differently distributed in the luminal (brush-border, BBM) and contraluminal (basolateral, BLM) membrane domains. A polar distribution of some, well characterized transporters of OA and OC in the rat PT cells is illustrated in Fig. 1C (OA transporters) and Fig. 1D (OC transporters).

SEX AND SPECIES DIFFERENCES IN THE EXPRESSION OF RENAL ORGANIC ANION TRANSPORTERS

Majority of the cloned and well-characterized OA transporters belong to the subfamily Slc22/SLC22 (Oat1/OAT1, Oat2/OAT2, Oat3/OAT3, OAT4, Oat5/OAT5,

etc...), which operate as anion exchangers (23), whereas the ATP-driven efflux pumps Mrp1, 3, 5, and 6, and Mrp2 and 4 belong to the subfamily Abcc/ABCC (24). Recent studies have shown that in the rodent and rabbit kidneys, some members of both transporter families exhibit species and/or sex differences in the expression of mRNA and/or protein, and in their localization along the nephron, and that these differences influence secretory functions of the organ. These differences are related to the sex hormone-regulated expression of specific transporters in one of the membrane domains of (largely) PT cells. From the available data for a limited number of renal OA transporters in rats, mice, rabbits, and humans, collected and shown in Table 2, one can conclude the following: (a) Oat1/OAT1 is always expressed in the PT BLM, but the male (M)-dominant sex differences in its expression are present in rats and mice, but not in rabbits and humans, (b) Oat2/OAT2 in rats and mice is localized to the PT BBM, where it exhibits the female (F)-dominant sex differences; its localization in rabbits is not known (at the level of mRNA, M = F), whereas in humans, this transporter is localized to the PT BLM and exhibits no sex differences, (c) Oat3/OAT3 in rodents and humans is localized to the PT BLM (in rabbits, the PT membrane domain has not been defined), but the expression is M-dominant in rats, F-dominant in mice, and sex-independent in rabbits and humans, (d) OAT4 is the human-specific transporter (not detected in rodents and rabbits) in the PT BBM, similarly expressed in M and F, (e) Oat5 is the rodent-specific transporter (not present in humans) in the PT BBM (and in intracellular organelles in mice), with F-dominant expression in both rats and

TABLE 2

Sex and species differences in the expression of several OA transporters in the mammalian kidneys.

Family	Transporter	Species and sex differences, localization in the nephron segment, membrane domain			
		Rats	Mice	Rabbits	Humans ^a
Slc/SLC	Oat1/OAT1	M > F	M > F	M = F	M = F
		PT, BLM	PT, BLM	PT, [?]	PT, BLM
	Oat2/OAT2	M < F	M < F	M = F ^b	M = F
		PT, BBM	PT, BBM	?	PT, BLM
	Oat3/OAT3	M > F	M < F ^b	M = F	M = F
		PT, BLM	PT, BLM	PT, [?]	PT, BLM
OAT4	ND	ND	ND	M = F PT, BBM	
Oat5	M < F	M < F	?	ND	
	PT, BBM	PT, BBM, IO	?	?	
Abc/ABC	Mrp4	M > F	M < F	?	?
			PT, BBM	PT, BBM	

Species-specific sex differences in the expression of OA transporters were observed at the level of protein and/or mRNA. For Oat1, Oat2, Oat3, and Oat5 expression in experimental animals, sex differences are determined by either stimulatory effects of androgens or inhibitory effects of estrogens and progesterone, or both, and are absent in prepubertal animals. Most data have been collected from various publications (15, 26–29). ^aOur unpublished data. ^bData from (29, 30), and our unpublished data. M, males; F, females; PT, proximal tubules; BBM, brush-border (luminal) membrane; BLM, basolateral (contraluminal) membrane; ^{IO}, intracellular organelles; ND, not detected in the species; ?, unknown.

TABLE 3

In rats, sex differences in the expression of some renal OA transporters correlate well with the excretion of relevant OA in urine.

Oat	Protein Expression	Organic anion	Urine excretion
Oat1	M > F	PAH, Furosemide	M > F
Oat2	M < F	PFOA	M < F
Oat3	M > F	PAH, Taurocholate	M > F

Data have been collected from the available literature and from own publications (for references see (15)). M, males; F, females; PAH, *p*-aminohippurate; PFOA, perfluorooctanoic acid.

mice, and (f) the expression of Mrp4 in the PT BBM is M-dominant in rats and F-dominant in mice; the data for rabbits and humans are not available. A number of other renal OATs in adult rats, mice, and in few other species, also exhibit sex differences in their expression at the level of protein and/or mRNA, whereas in prepubertal animals, the expression of thus far tested OA transporters was low and sex-independent (15, 25).

As has been tested in rats, mice and rabbits, the urine excretion of relevant OA correlates well with the renal expression of Oats. Thus, sex differences in the renal expression of Oat1, Oat2, and Oat3 in adult rats clearly correlate with similar differences in the urine excretion of their substrates (Table 3), whereas in the adult rabbits, in which the expression of these Oats is similar in both sexes, the excretion of relevant OA is also similar (30).

Furthermore, the prepubertal M and F rats excrete OA with similar and much lower rate than the adults, which is in good correlation with the sex-independent and much lower expression of renal Oats (for references see (15)). In addition, in mice with the knocked-out Oat1 and Oat3 genes, the urine excretion of *p*-aminohippurate (PAH) and a few other OA is strongly impaired (29, 31, 32). Since the localization and the level of protein expression in the cell membrane is one of the major determinants (next to the substrate specificity and kinetic characteristics) of the transporter function, the observations in humans that: a) none of the indicated transporters shows sex differences in the expression, b) OAT2 is localized in the membrane domain (BLM) which is opposite from that in rodents (BBM), and c) OAT4 is present only in humans, whereas Oat5 is rodent-specific, indicate that the renal handling of OA with the all possible consequences, such as interactions and nephrotoxicity of anionic drugs, may be in humans different from that in rodents and rabbits. Moreover, different sex-related expression of Oat3 in rats, mice, and rabbits, and of Mrp4 in rats and mice, indicates that the overall handling and secretion of many OA may be different among these species. The respective transport studies in humans are scarce; only a few of them have shown that the renal clearance of some drugs and/or their metabolites may be sex-related, but these differences may rather reflect the sex-depend-

ent metabolism of anionic (and other) drugs catalyzed by drug-metabolizing enzymes, not the transporters-mediated active secretion in the renal tubules (3, 15).

SEX AND SPECIES DIFFERENCES IN THE EXPRESSION OF RENAL ORGANIC CATION TRANSPORTERS

A number of OC transporters from different protein families and species have been cloned and characterized, and their functional roles have been studied mainly in the liver and kidneys (8). The most important OC transporters are grouped into the families Slc22/SLC22 (Oct1/OCT1, Oct2/OCT2, Oct3/OCT3, Octn1/OCTN1, Octn2/OCTN2), Slc47/SLC47 (MATE1, MATE2, MATE2-K), and Abcb/ABCB (Mdr1/MDR1 (P-glycoprotein, P-gp)). Oct1-3/OCT1-3 represent polyspecific bidirectional transporters that mediate electrogenic, sodium- and pH-independent intracellular uptake of OC *via* facilitated diffusion. In the rat PT, these transporter are predominantly localized to the BLM, where they mediate the first step of the renal OC excretion. The second, exit step across the BBM is largely mediated by the electroneutral H⁺-OC exchangers MATE1 (in rodents, rabbits and humans), MATE2 (in mice, not known for rats, not present in rabbits and humans), and MATE2-K (in rabbits and humans, not present in rats and mice), and by the ATP-driven efflux pump Mdr1/MDR1. Besides in expression and localization, OC transporters in various species differ in substrate specificity, inhibitor sensitivity, transport mechanism, and regulation (8, 33).

The renal OC transporters have been extensively studied recent years, but most of this research showed their expression at the level of mRNA, whereas only a few transporters were described also at the protein level. As listed in Table 4, the mRNA expression of various renal OC transporters in different species exhibits different pattern of sex dependency. Thus, in the rat, mouse, rabbit and human kidneys: (a) mRNA expression of various OC transporters is species-dependent, but sex differences are present only in some cases, (b) expression of both mRNA and protein have been thus far studied and correlated only in a few cases, (c) in the Oct/OCT subfamily, rats and mice express the Oct1 mRNA in similar abundance in M and F, whereas the protein is localized to the proximal tubule BLM with (largely) M-dominant expression. However, rabbits do not express the Oct1 mRNA and protein at all, whereas in humans, OCT1 is localized to the apical membrane of proximal and distal tubules with similar expression in M and F. (d) The expression of renal Oct2/OCT2 in different species has been studied in more detail at both mRNA and protein level. The expression of Oct2 mRNA in rats, mice and rabbits exhibits the M-dominant pattern, but at the protein level this basolateral transporter in PT is clearly stronger in M than F rats and mice, but sex-independent in rabbits and humans. (e) In the Octn/OCTN subfamily, the mRNA expression of the indicated transporters is either sex-independent or still controversial, whereas the Octn2 protein in rats and mice is localized to the PT BBM. (f) In the

TABLE 4

Sex and species differences in the renal expression of OC transporters at the level of mRNA and/or protein.

OC Transporter	Species	mRNA M vs. F	Protein (Nephron segment, Membrane domain)	M vs. F
Oct1/OCT1	Rat	M ≤ F	PT, BLM	M ≥ F
	Mouse	M = F	PT, BLM	M > F
	Rabbit	ND	ND	ND
	Human	+/?	PT & DT, AM	M = F
Oct2/OCT2	Rat	M > F	PT, BLM	M > F
	Mouse	M > F	PT, BLM	M > F
	Rabbit	M > F	PT, ?	M = F
	Human	+/?	PT, BLM	M = F
Oct3/OCT3	Rat	M = F	?	
	Mouse	M = F	?	
	Rabbit	M = F	?	
	Human	+/?	?	
Octn1/OCTN1	Rat	M = F	?	
	Mouse	M = F	?	
	Rabbit	+/?	?	
	Human	+/?	?	
Octn2/OCTN2	Rat	M ≥ F	PT, BBM	
	Mouse	M = F	PT, BBM	
	Rabbit	+/?	?	
	Human	+/?	?	
MATE1	Rat	M > F	PT, BBM	M > F
	Mouse	M > F	Various segments, AM	?
	Rabbit	M = F	?	
	Human	+/?	PT, BBM	?
MATE2	Rat	+/?	?	
	Mouse	M = F		
	Rabbit	ND	ND	
	Human	ND	ND	
MATE2-K	Rat	ND	ND	
	Mouse	ND	ND	
	Rabbit	M = F	?	
	Human	+/?	PT, BBM	?
Mdr1a	Rat	M > F	?	
	Mouse	M ≤ F	?	
Mdr1b	Rat	+/?	?	
	Mouse	M < F	?	
Mdr2	Rat	+/?	?	
	Mouse	M = F	?	
MDR1	Human	+/?	?	

Data have been collected from the previously reviewed literature (15), from other studies (34, 36–49), and from own unpublished studies. The mRNA expression was determined in the kidney cortex or whole kidney tissue, whereas protein expression was determined by immunocytochemistry in tissue cryosections and/or by Western blotting in cell membranes isolated from various kidney zones. F, females; M, males; ND, not detected; +/?, mRNA detected, but sex-dependency unknown; ?, unknown data; PT, proximal tubules; BLM, basolateral membrane; DT, distal tubules; AM, apical membrane.

MATE subfamily, MATE1 in the rat kidney is localized to the PT BBM and M-dominant in both mRNA and protein expression. Mice exhibit higher expression of mRNA in M, but the protein was detected in the apical membrane of various tubule segments with unknown levels of expression in M and F. MATE1 is present also in the rabbit and human kidneys, but in these species the levels of MATE1 mRNA and protein expression in M and F has been poorly investigated. However, clear species differences exist in the expression of MATE2, which is present in mice (not clear in rats), but not in rabbits and humans, and MATE2-K, which is present in rabbits and humans, but not in rodents. (g) In the Mdr/MDR subfamily, the Mdr1a mRNA expression in the rat kidneys is M > F, whereas in mice, the mRNA expression of this transporters may be just opposite, e.g., M < F. Where tested, sex hormones responsible for the observed sex differences in the expression of mRNA and/or protein of the specific OC transporters have been defined, whereas in prepubertal animals, the expression of both parameters is low and sex-independent (reviewed in (15)).

Several *in vivo* studies in variously-treated rats and mice, and/or *in vitro* studies in tissue slices or isolated renal BLM vesicles from the same animals, have correlated the protein expression of some OC transporters and the rates of OC secretion (34, reviewed in (15)). The data have shown that: (a) M rats and mice exhibit higher rate of OC tetraethylammonium (TEA) clearance than the F animals, (b) in rodents, tissue slices from the M kidneys exhibit higher accumulation of TEA than the slices from the F kidneys, (c) in BLM vesicles isolated from the rat kidneys, TEA uptake in the vesicles from M kidneys is greater than in the vesicles from F kidneys, (d) gonadectomy of M rodents downregulates, the treatment of these animals with testosterone strongly upregulates, whereas the treatment with estradiol slightly downregulates the renal expression of Oct2 protein and the accumulation of TEA in kidney tissue slices. These data thus indicate the major role of androgens in regulating the Oct2-mediated OC secretion in rodents. An important role of this transporter for OC secretion in the mouse kidneys can be further demonstrated in the animals deficient (knock-out) in Oct1 and Oct2, in which the renal secre-

TABLE 5

Species differences in the rates of OA transport, affinity for OC substrates, inhibitory constant of OC transport, and inhibition of an OC transport with other OC in various experimental models.

Parameter	Experimental model	Species differences
Substrate specificity:		
Quinidine transport	Oct1/OCT1-transfected XO	rat -, human +
Na ⁺ -L-carnitine cotransport	Octn1/OCTN1-transfected cells	rat -, mouse +, human +
Relative rate of transport:		
TEA uptake	Octn2/OCTN2-transfected cells	rat > mouse > human
Na ⁺ -L-carnitine cotransport	Octn2/OCTN2-transfected cells	rat < mouse < human
K_m value for:		
Choline	Oct2/OCT2-transfected XO	rat > human
TEA (Octn1-mediated)	Renal cortical BBMV	rat > rabbit
K_i value for:		
Inhibition of MPP uptake by n-TAA	Oct1/OCT1-transfected XO	rat, mouse, rabbit < human
Inhibition of TEA transport by various OC	Oct1/OCT1-transfected cells	rat < human
	Oct2/OCT2-transfected cells	rat > human
IC₅₀ value for:		
Inhibition of TEA uptake by TBA, TPA, cimetidine guanidine and famotidine	Oct2/OCT2-transfected cells	rabbit < human
Inhibition of TEA uptake by tyramine, carbachol and choline	Oct2/OCT2-transfected cells	rabbit > human
Inhibition of OC transport with other OC:		
Inhibition of L-carnitine transport by TEA and choline	Octn2/OCTN2-transfected cells	rat > human
Inhibition of TEA transport by cimetidine and rhodamine 123	MATE1-transfected cells	mouse > human
Inhibition of TEA transport by quinidine, verapamil, nicotine, corticosterone, testosterone and quercetin	MATE1-transfected cells	mouse < human

Data have been collected from the literature (7, 42, 50–62). XO, Xenopus oocytes; Transfected cells, various kinds of cultured cells transfected with the indicated OC transporters; BBMV, isolated brush-border membrane vesicles; (-) Absence or (+) presence of transport. K_m, Michaelis constant. MPP, 1-methyl-4-phenylpyridinium; n-TAA, *n*-tetraalkylammonium compounds; TEA, tetraethylammonium; TBA, tetrabutylammonium.

TABLE 6

Species differences in short term regulation of the mammalian OC transporters.

Species & Experimental model	Transport	Effector	Effect on transport
Rat			
Oct1-transfected HEK-293 cells	ASP ⁺ uptake	PKA activation	Increase
		PKC activation	Increase
Rabbit			
Oct2-transfected CHO-K1 cells	TEA uptake	PKA activation	Increase
Isolated PT segments	TEA uptake	PKA activation	Increase
Human			
OCT1-transfected HEK-293 cells	Amiloride & ASP ⁺ uptake	PKA activation	Decrease
		PKC activation	Decrease/NE
OCT2-transfected HEK-293 cells	Amiloride & ASP ⁺ uptake	PKA activation	Decrease
		PKC activation	Decrease/NE
Isolated PT segments	ASP ⁺ uptake	PKC activation	Decrease

Data have been collected from the literature (33, 63–69). ASP⁺, fluorescent cationic dye 4-(4-dimethylamino)styryl-N-methylpyridinium; TEA, tetraethylammonium; PT, proximal tubules; PKA, protein kinase A; PKC, protein kinase C; NE, no effect.

tion of OA is nearly abolished (35). However, the studies in isolated PT from the M and F rabbit kidneys have shown similar uptake of TEA in both sexes, which compares to similar and sex-independent expression of Oct2 protein in their kidneys (30).

Species differences in some OC transporters have also been demonstrated concerning substrate selectivity and transport rates, sensitivity to inhibitors, and regulation. A comparison of kinetic characteristics of various OC transporters from different species have been tested largely following their expression in *Xenopus* oocytes and cultured cells. The data collected from the current literature clearly indicate species differences in many characteristics among the comparable OC transporters in rats, mice, rabbits and humans. As listed in Table 5, the related OC transporters in rodents, rabbits and humans differ in substrate specificity, relative transport rate with specific substrates, affinity (K_m) for specific substrates, and inhibition of the transporter activity with various substrates (K_i and IC_{50} values, inhibition of the OC transport with other OCs). Overall, these data indicate that the OC transporters from each subfamily exhibit species differences in a variety of kinetic characteristics that may result in different, species-specific functional performance in the mammalian kidneys and other organs.

Various aspects of long-term (developmental, hormonal and nutritional regulation, regulation under pathological conditions) and short-term regulation of OC transport and expression and/or activity of various OC transporters in the mammalian kidneys *in vivo* and in various experimental models *in vitro*, have been recently reviewed (33). A set of information that point to species differences in short-term regulation of the activity of some of these transporters has been collected from the literature and summarized in Table 6. Most of these information have been generated in the cell lines transfected with the

defined animal or human OC transporters, and in some cases the regulation of their activity (transporter-mediated uptake of an OC) in the cells could be correlated with the relevant transport in PT segments isolated from the same species. Thus (Table 6), (a) in the cells transfected with the rat Oct1, activation of protein kinases A (PKA) and C (PKC) resulted in upregulation of the OC transport, whereas in the same cell line transfected with the human OCT1, activation of these enzymes caused downregulation of the OC transport (in one study the effect was not observed), (b) in the cells transfected with the rabbit Oct2, activation of PKA activity caused upregulation of the OC uptake, and the same effect was present in the isolated rabbit PT segments, whereas (c) in the cells transfected with the human OCT2, and in isolated human PT segments, the activation of both kinases down-regulated the OC transport. These experiments *in vitro* indicate that the short-term regulation of OC transporter activity in the mammalian kidneys may be species-specific also *in vivo*.

CONCLUSION

In rodents, various renal transporters of OA and OC exhibit sex differences in their protein (and mRNA) expression and functional characteristics. This phenomenon may be relevant during life in these animals in conditions that are associated with significant changes in blood levels of sex hormones (female hormonal cycle/oestrus in rodents, pregnancy, ageing). When compared in rats, mice, rabbits and humans, some renal OA and OC transporters also exhibit species differences in their presence, expression level, sex-dependence, membrane domain-specific localization in the cells, various kinetic characteristics, and regulation. Humans exhibit no sex differences in the expression of thus far tested OA and OC transporters, and other characteristics related to the-

se transporters are in many respects different from those in rodents and rabbits. Although in humans sex differences in pharmacokinetics have been identified for some drugs, they are small, and their clinical relevance is minor (70). Therefore, (a) data on OA and OC transport and transporters in one species can not simply be regarded as relevant for other species, and (b) physiological, pharmacological and toxicological data in rats, mice, and rabbits, that are related to the functions of these transporters in the kidneys, may not be relevant to the situation in humans.

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