PERIODICUM BIOLOGORUM
VOL. 113, No 1, 7–16, 2011

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Leading article

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

IVAN SABOLIĆ
DAVORKA BRELJAK
MARIJA LJUBOJEVIĆ
HRVOJE BRZICA

Unit of Molecular Toxicology
Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb, Croatia

Correspondence:
Ivan Sabolić
Unit of Molecular Toxicology
Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb,Croatia
E-mail: sabolic@imi.hr

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

Key words: experimental animals, gender differences, mammalian kidney, nephrotoxicity, organic anions, organic cations, proximal tubule

Received February 21, 2011.

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 disfunctional 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-bread 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 licenced 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, efficasy 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 reviewes *(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 transported 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-H2-receptor antagonists); **Conjugates** (steroid hormones conjugated with sulfate, cysteine, glycine, and glucuronide); **Diagnostic and experimental drugs** (p-aminohippuric 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, anestetics, 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)*.

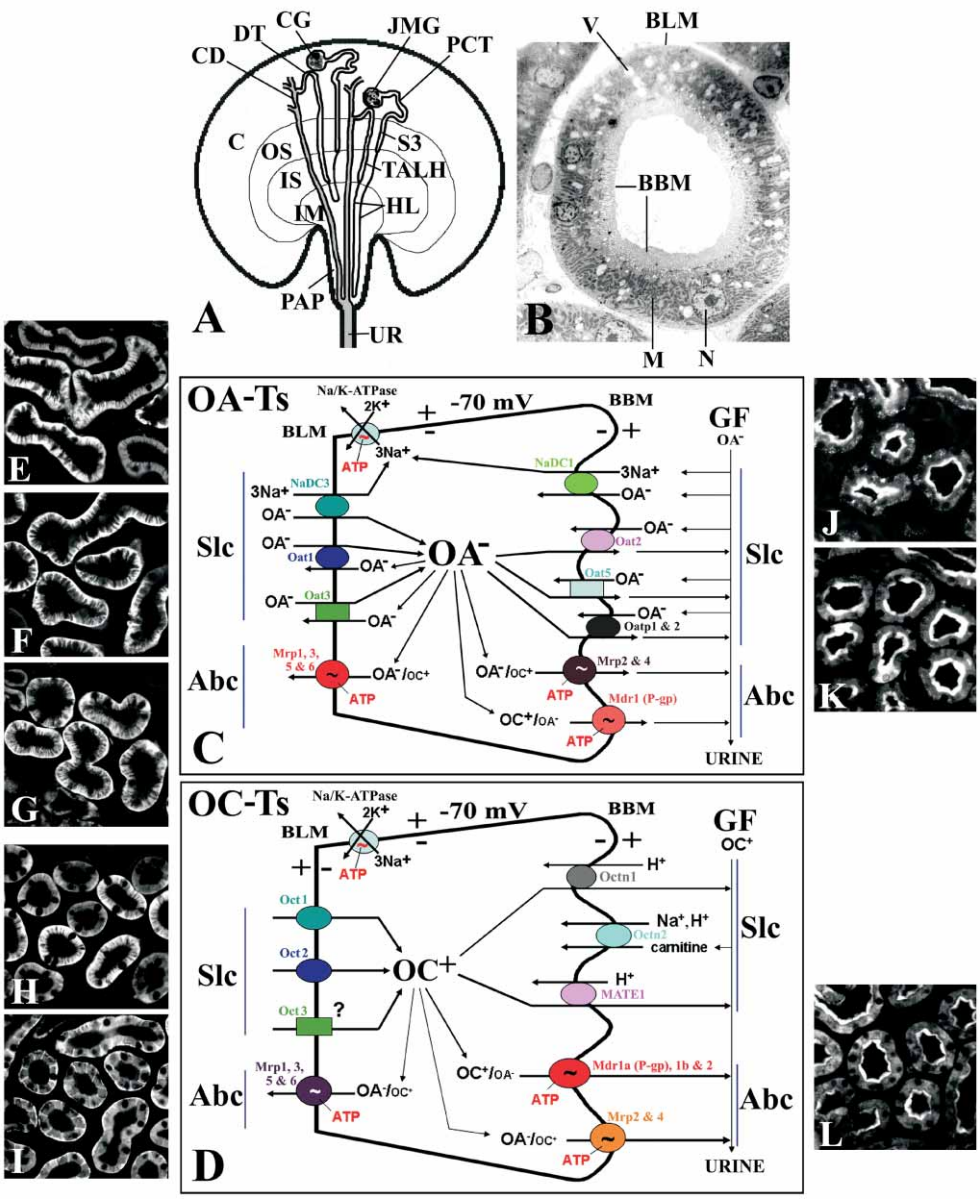

Figure 1. *Polar distribution of the common drug transporters in the rat PT cells. A. Definition of various nephron segments in relation to specific tissue zones. C, cortex; OS, outer stripe; IS, inner stripe; IM, inner medulla; PAP, papilla; UR, ureter; CG, cortical glomerulus; JMG, juxtamedullary glomerulus; 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 terciary-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 casette) 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 Octn1 (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 Octn2. 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, Oct1; I, Oct2; J, Oat2; K, Oat5; L, Mdr1.*

bers of the ATP-binding casette (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 iatrogen (medicaments/drugs) or alimentary (food constituents) reasons, or as the enviromental 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.

		Species and sex differences, localization in the nephron segment, membrane domain			
Family	**Transporter**	**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)*. [a]Our unpublished data. [b]Data 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-dependent metabolism of anionic (and other) drugs catalized 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 grupped 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 expresssion 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 defficient (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
Ki 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
IC_{50} 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 &	PKA activation	Decrease
	ASP^+ uptake	PKC activation	Decrease/NE
OCT2-transfected HEK-293 cells	Amiloride &	PKA activation	Decrease
	ASP^+ uptake	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, tetraetylammonium; 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 patological 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 downregulated 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.

Acknowledgement: *This work was supported by grant No. 022-0222148-2146 (Mammalian Renal Transporters; Gender Differences and Effects of Toxic Metals) from the Ministry of Science, Education and Sports, Republic of Croatia (I.S.).*

REFERENCES

1. ANZAI N, KANAI Y, ENDOU H 2006 Organic anion transporter family: Current knowledge. *J Pharmacol Sci 100:* 411–426
2. EL-SHEIKH A A K, MASEREEUW R, RUSSEL F G M 2008 Mechanisms of renal anionic drug transport. *Eur J Pharmacol 585:* 245–255
3. FRANCONI F, BRUNELLESCI S, STEARDO L, CUOMO V 2007 Gender differences in drug responses. *Pharmacol Res 55:* 81–95
4. GIACOMINI K M, HUANG S M, TWEEDIE D J, BENET L Z, BROUWER K L, CHU X, DAHLIN A, EVERS R, FISCHER V, HILLGREN K M, HOFFMASTER K A, ISHIKAWA T, KEPPLER D, KIM R B, LEE C A, NIEMI M, POLLI J W, SUGIYAMA Y, SWAAN P W, WARE J A, WRIGHT S H, YEE S W, ZAMEK-GLISZCZYNSKI M J, ZHANG L 2010 Membrane transporters and drug development. *Nat Rev Drug Discov 9:* 215–236
5. HO R H, KIM R B 2005 Transporters and drug therapy: Implications for drug disposition and disease. *Clin Pharmacol Ther 78:* 260–277
6. KOEPSELL H 2004 Polyspecific organic cation transporters: their functions and interactions with drugs. *Trends Pharmacol Sci 25:* 375–381
7. KOEPSELL H, GORBOULEV V, ARNDT P 1999 Molecular pharmacology of organic cation transporters in kidney. *J Membrane Biol 167:* 103–117
8. KOEPSELL H, SCHMITT B M, GORBOULEV V 2003 Organic cation transporters. *Rev Physiol Biochem Pharmacol 150:* 1–35
9. LAUNAY-VACHER V, IZZEDINE H, KARIE S, HULOT JS, BAUMELOU A, DERAY G 2006 Renal tubular drug transporters. *Nephron Physiol 103:* 97–p106
10. LEE W, KIM R B 2004 Transporters and renal drug elimination. *Ann Rev Pharmacol Toxicol* 44: 137–166
11. LI M, ANDERSON G D, WANG J 2006 Drug-drug interactions involving membrane transporters in the human kidney. *Expert Opin Drug Metab Toxicol 2:* 505–532
12. MORRIS M E, LEE H J, PREDKO L M 2003 Gender differences in the membrane transport of endogenous and exogenous compounds. *Pharmacol Rev 55:* 229–240
13. RIZWAN A N, BURCKHARDT G 2007 Organic anion transporters of the SLC22 family: biopharmaceutical, physiological, and pathological roles. *Pharmaceut Res 24:* 450–470
14. ROBERTSON E E, RANKIN G O 2006 Human renal organic anion transporters: Characteristics and contributions to drug and drug metabolite excretion. *Pharmacol Ther 109:* 399–412
15. SABOLIC I, ASIF A R, BUDACH W E, WANKE C, BAHN A, BURCKHARDT G 2007 Gender differences in kidney function. *Pfluegers Arch Eur J Physiol 455:* 397–429
16. SEKINE T, CHA S H, ENDOU H 2000 The multispecific organic anion transporter (OAT) family. *Pfluegers Arch Eur J Physiol 440:* 337–350
17. SEKINE T, MIYAZAKI H, ENDOU H 2006 Molecular physiology or renal organic anion transporters. *Am J Physiol Renal Physiol 290:* F251–F261
18. SRIMAROENG C, PERRY L J, PRITCHARD J B 2008 Physiology, structure, and regulation of the cloned organic anion transporters. *Xenobiotica 38:* 889–935
19. SWEET D H 2005 Organic anion transporter (Slc22a) family members as mediators of toxicity. *Toxicol Appl Pharmacol 204:* 198–215
20. TERLOW S A, MASEREEUW R, RUSSEL F G M 2003 Modulatory effects of hormones, drugs, and toxic events on renal organic anion transport. *Biochem Pharmacol 65:* 1393–1405
21. VAN AUBEL R A M H, MASEREEUW R, RUSSEL F G M 2000 Molecular pharmacology of renal organic anion transporters. *Am J Physiol Renal Physiol 279:* F216–F232
22. WRIGHT S H 2005 Role of organic cation transporters in the renal handling of therapeutic agents and xenobiotics. *Toxicol Appl Pharmacol 204:* 309–319
23. HEDIGER M A (Guest Editor) 2004 Special issue: The ABCs of solute carriers: physiological, pathological, and therapeutic implications of human membrane transport proteins. *Pflügers Arch Eur J Physiol 447 (5)*
24. STIEGER B, HIGGINS C F (Guest Editors) 2007 Special issue: Twenty years of ABC transporters. *Pflügers Arch Eur J Physiol 453 (5)*
25. BRZICA H, BRELJAK D, KRICK W, LOVRIC M, BURCKHARDT, G, BURCKHARDT B C, SABOLIC I 2009 The liver and kidney expression of sulfate anion transporter sat-1 in rats exhibits male-dominant gender differences. *Pflügers Arch Eur J Physiol 457:* 1381–1392
26. BRELJAK D, LJUBOJEVIC M, BALEN D, ZLENDER V, BRZICA H, MICEK V, KUSAN M, ANZAI N, SABOLIC I 2010 Renal expression of organic anion transporter Oat5 in rats and mice exhibits the female-dominant sex differences. *Histol Histopathol 25:* 1385–1402
27. LJUBOJEVIC M, BALEN D, BRELJAK D, KUSAN M, ANZAI N, BAHN A, BURCKHARDT G, SABOLIC I 2007 Renal expression of organic anion transporter OAT2 in rats and mice is regulated by sex hormones. *Am J Physiol Renal Physiol 292:* F361–F372
28. LJUBOJEVIC M, HERAK-KRAMBERGER C M, HAGOS Y, BAHN A, ENDOU H, BURCKHARDT G, SABOLIC I 2004 Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. *Am J Physiol Renal Physiol 287:* F124–F138
29. VAN WERT A L, BAILEY R M, SWEET D H 2007 Organic anion transporter 3 (Oat3/*Slc22a8*) knockout mice exhibit altered clearance and distribution of penicillin G. *Am J Physiol Renal Physiol 293:* F1332–F1341
30. GROVES C E, SUHRE W B, CHERRINGTON N J, WRIGHT S H 2006 Sex differences in the mRNA, protein, and functional expression of organic anion transporter (Oat) 1, OAT3, and organic cation transporter (OCT) 2 in rabbit renal proximal tubules. *J Pharmacol Exp Therap 316:* 743–752
31. ERALY S A, VALLON V, VAUGHAN D A, GANGOITI J A, RICHTER K, NAGLE M, MONTE J C, RIEG T, TRUONG D M, LONG J M, BARSHOP B A, KALER G, NIGAM S K 2006 Decreased renal organic anion secretion and plasma accumulation of endogenous organic anions in OAT1 knock-out mice. *J Biol Chem 281*: 5072–5083
32. SWEET D H, MILLER D S, PRITCHARD J B, FUJIWARA Y, BEIER D R, NIGAM S K 2002 Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8)) knockout mice. *J Biol Chem 277:* 26934–26943
33. CIARIMBOLI G, SCHLATTER E 2005 Regulation of organic cation transport. *Pflugers Arch Eur J Physiol 449:* 423–441
34. MEETAM P, SRIMAROENG C, SOODVILAI S, CHATSUDTHIPONG V 2009 Regulatory role of testosterone in organic cation transport: in vivo and in vitro studies. *Biol Pharm Bull 32:* 982–987
35. JONKER J W, WAGENAAR E, VAN EIJL S, SCHINKEL A H 2003 Defficiency in the organic cation transporters 1 and 2 (Oct1/Oct2 [Slc22a1/Slc22a2]) in mice abolishes renal secretion of organic cations. *Mol Cell Biol 23:* 7902–7908
36. CUI Y J, CHENG X, WEAVER Y M, KLAASSEN C D 2009 Tissue distribution, gender-divergent expression, ontogeny, and chemical induction of multidrug resistance transporter genes (*Mdr1a*, *Mdr1b*, *Mdr2*) in Mice. *Drug Metab Dispos 37:* 203–210
37. KARBACH U, KRICKE J, MEYER-WENTRUP F, GORBOULEV V, VOLK C, LOFFING-CUENI D, KAISSLING B, BACH-

MANN S, KOEPSELL H 2000 Localization of organic cation transporters OCT1 and OCT2 in rat kidney. *Am J Physiol Renal Physiol 279:* F679–F687

38. LICKTEIG A J, CHENG X, AUGUSTINE L M, KLAASSEN C D, CHERRINGTON N J 2008 Tissue distribution, ontogeny and induction of the transporters multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. *Life Sci 83:* 59–64
39. LU H, KLAASSEN C D 2008 Gender differences in mRNA expression of ATP-binding cassette efflux and bile acid transporters in kidney, liver, and intestine of 5/6 nephrectomized rats. *Drug Metab Dispos 36:* 16–23
40. MASUDA S, TERADA T, YONEZAWA A, TANIHARA Y, KISHIMOTO K, KATSURA T, OGAWA O, INUI K 2006 Identification and functional characterization of a new human kidney–specific H^+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. *J Am Soc Nephrol 17:* 2127–2135
41. NISHIHARA K, MASUDA S, JI L, KATSURA T, INUI K 2007 Pharmacokinetic significance of luminal multidrug and toxin extrusion 1 in chronic renal failure rats. *Biochem Pharmacol 73:*1482–1490
42. OTSUKA M, MATSUMOTO T, MORIMOTO R, ARIOKA S, OMOTE H, MORIYAMA Y 2005 A human transporter protein that mediates the final excretion step for toxic organic cations. *PNAS 102:* 17923–17928
43. SLITT AL, CHERRINGTON NJ, HARTLEY DP, LEAZER TM, KLAASSEN C D 2002 Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. *Drug Metab Disp 30:* 212–219
44. TERADA T, MASUDA S, ASAKA J, TSUDA M, KATSURA T, INUI K 2006 Molecular cloning, functional characterization and tissue distribution of rat H^+/organic cation antiporter MATE1. *Pharm Res 23:* 1696–1701
45. TZVETKOV M V, VORMFELDE S V, BALEN D, MEINEKE I, SCHMIDT T, SEHRT D, SABOLIC I, KOEPSELL H, BROCKMOLLER J 2009 The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. *Clin Pharmacol Therap 86:* 299–306
46. URAKAMI Y, OKUDA M, SAITO H, INUI K 2000 Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett 473:* 173–176
47. WRIGHT S H, EVANS K K, ZHANG X, CHERRINGTON N J, SITAR D S, DANTZLER W H 2004 Functional map of TEA transport activity in isolated rabbit renal proximal tubules. *Am J Physiol Renal Physiol 287:* F442–F451
48. ZHANG X, CHERRINGTON N J, WRIGHT S H 2007 Molecular identification and functional characterization of rabbit MATE1 and MATE2-K. *Am J Physiol Renal Physiol 293:* F360–F370
49. ZHANG X, EVANS K K, WRIGHT S H 2002 Molecular cloning of rabbit organic cation transporter rbOCT2 and functional comparisons with rbOCT1. *Am J Physiol Renal Physiol 283:* F124–F133
50. DRESSER M J, GRAY A T, GIACOMINI K M 2000 Kinetic and selectivity differences between rodent, rabbit, and human organic cation transporters (OCT1). *J Pharmacol Exp Ther 292:* 1146–1152
51. HIASA M, MATSUMO T, KOMATSU T, MORIYAMA Y 2006 Wide variety of locations for rodent MATE1, a transporter protein that mediates the final excretion step for toxic organic cations. *Am J Physiol Cell Physiol 291:* C678–C686
52. NAGEL G, VOLK C, FRIEDRICH T, ULZHEIMER JC, BAMBERG E, KOEPSELL H 1997 A reevaluation of substrate specificity of the rat cation transporter rOCT1. *J Biol Chem* 272: 31953–31956
53. SCHALI C, SCHILD L, OVERNEY J, ROCH-RAMEL F 1983 Secretion of tetraethylammonium by proximal tubules of rabbit kidneys. *Am J Physiol Renal Physiol 245:* F238–F246
54. SUHRE W M, EKINS S, CHANG C, SWAAN P W, WRIGHT S H 2005 Molecular determinants of substrate/inhibitor binding to the human and rabbit renal organic cation transporters hOCT2 and rbOCT2. *Mol Pharmacol 67:* 1067–1077
55. SWEET D H, BUSH K T, NIGAM S K 2001 The organic anion transporter family: from physiology to ontogeny and the clinic. *Am J Physiol Renal Physiol 281:* F197–F205
56. SWEET D H, MILLER D S, PRITCHARD J B 2001 Ventricular choline transport. A role for organic cation transporter 2 expressed in choroid plexus. *J Biol Chem 276:* 41611–41619
57. TAKANO M, INUI K, OKANO T, SAITO H, HORI R 1984 Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim Biophys Acta 773:* 113–24
58. TAMAI I, OHASHI R, NEZU J, SAI Y, KOBAYASHI D, OKU A, SHIMANE M, TSUJI A 2000 Molecular and functional characterization of organic cation/carnitine transporter family in mice. *J Biol Chem 275:* 40064–40072.
59. WRIGHT S H, WUNZ T M 1987 Transport of tetraethylammonium by rabbit renal brush-border and basolateral membrane vesicles. *Am J Physiol Renal Physiol 253:* F1040–F1050
60. WU X, GEORGE R L, HUANG W, WANG H, CONWAY S J, LEIBACH F H, GANAPATHY V 2000 Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim Biophys Acta 1466:* 315–327
61. WU X, HUANG W, PRASAD P D, SETH P, RAJAN D P, LEIBACH F H, CHEN J, CONWAY S J, GANAPATHY V 1999 Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J Pharmacol Exp Ther 290:* 1482–1492
62. YABUUCHI H, TAMAI I, NEZU J, SAKAMOTO K, OKU A, SHIMANE M, SAI Y, TSUJI A 1999 Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J Pharmacol Exp Ther 289:* 768–773
63. BIERMANN J, LANG D, GORBOULEV G, KOEPSELL H, SINDIC A, SCHRÖTER R, ZVIRBLIENE A, PAVENSTÄDT H, SCHLATTER E, CIARIMBOLI G 2006 Characterization of regulatory mechanisms and states of human organic cation transporter 2. *Am J Physiol Cell Physiol 290:* C1521–C1531
64. ÇETINKAYA I, CIARIMBOLI G, YALÇINKAYA G, MEHRENS T, VELIC A, HIRSCH J R, GORBOULEV V, KOEPSELL H, SCHLATTER E 2003 Regulation of human organic cation transporter hOCT2 by PKA, PI3K, and calmodulin-dependent kinases. *Am J Physiol Renal Physiol 284:* F293–F302
65. CIARIMBOLI G, STRUWE K, ARNDT P, GORBOULEV V, KOEPSELL H, SCHLATTER E, HIRSCH J R 2004 Regulation of the human organic cation transporter hOCT1. *J Cell Physiol 201:* 420–428
66. HOHAGE H, MORTH D M, QUERL I U, GREVEN J 1994 Regulation by PKC of the contraluminal transport system for organic cations in rabbit kidney S2 proximal tubules. *J Pharmacol Exp Ther 268:* 897–901
67. MEHRENS T, LELLECK S, ÇETINKAYA I, KNOLLMANN M, HOHAGE H, GORBOULEV V, BOKNIK P, KOEPSELL H, SCHLATTER E 2000 The affinity of the organic cation transporter rOCT1 is increased by protein kinase C-dependent phosphorylation. *J Am Soc Nephrol 11:* 1216–1224
68. PIETIG G, MEHRENS T, HIRSCH J R, ÇETINKAYA I, PIECHOTA H, SCHLATTER E 2001 Properties and regulation of organic cation transport in freshly isolated human proximal tubules. *J Biol Chem 276:* 33741–33746
69. SOODVILAI S, CHATSUDTHIPONG A, CHATSUDTHIPONG V 2007 Role of MAPK and PKA in regulation of rbOCT2-mediated renal organic cation transport. *Am J Physiol Renal Physiol 293:* F21–F27
70. MEIBOHM B, BEIERLE I, DERENDORF H 2002 How important are gender differences in pharmacokinetics? *Clin Pharmacokinet 41*: 329–342