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Topical Review

Molecular Pharmacology of Organic Cation Transporters in Kidney

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Introduction

The homeostasis of endogenous organic cations, such as choline and N^1 P-methylnicotinamide, monoamine neurotransmitters, cationic drugs, such as cimetidine, morphine, quinine and amantadine, and of cationic xenobiotics is controlled by reabsorption and excretion in the small intestine, by metabolic conversion and excretion in the liver, and by reabsorption and excretion in the kidney [1–6]. In the kidney organic cations may be ultrafiltrated in the glomeruli and reabsorbed or secreted in renal tubules. Hydrophilic cations are readily ultrafiltrated, however, more hydrophobic cations are bound to plasma membrane proteins that may not permeate the filtration barrier. Secretion and reabsorption of organic cations have been described in renal proximal tubules but may also occur in distal tubules or collecting ducts [7–11]. These processes have been functionally characterized by measurements using intact kidneys or tissue slices [10, 12–14], by microperfusion experiments using proximal tubules [7, 9, 15], by uptake measurements using isolated tubules or cells [8, 11, 16, 17], and by uptake measurements using membrane vesicles from proximal tubules [18–28]. In luminal and basolateral membranes of proximal tubules different transport processes have been demonstrated, although all the involved transport systems may not have been identified by these measurements because a variety of different polyspecific organic cation transporters with overlapping substrate specificity are expressed [5]. After the cloning of the first organic cation transporter *rOCT1* from a rat kidney library [29], an increasing number of homologous cation transporters have been identified and it has been shown that the renal anion transporters also belong to this new protein family. The functional characterization of the expressed transporters and their immunohistochemical localization in the kidney has begun [30–35]. This review describes the molecular structure of renal organic cation transporters and homologous gene products. The transport properties and renal localization of the cloned cation transporters are summarized and their presumed role in renal cation handling of endogenous and exogenous cations is discussed.

Functional Characterization of Organic Cation Transport in Kidney

Renal cation transport in proximal tubules, distal tubules and collecting ducts has been investigated in various species [3–5, 11, 36, 37]. In the nephron organic cations may be secreted or reabsorbed depending on the cation concentration in the plasma. Both processes may occur in the same nephron segment or in different parts of the nephron. Most knowledge regarding cation uptake is based upon information obtained from renal proximal tubules from the rat or rabbit employing microperfusion experiments, uptake measurements using isolated tubules and uptake measurements using membrane vesicles. These methods allowed separate uptake measurements over the luminal or basolateral membrane. Heterogeneity in cation transport has been observed in the different segments of the proximal tubules [8, 9]. In the S1 segments, where the highest cation secretion was observed, three main cation transport systems have been function-

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Key words: Cation excretion — Cation reabsorption — Kidney — Polyspecific transporters — OCT-family

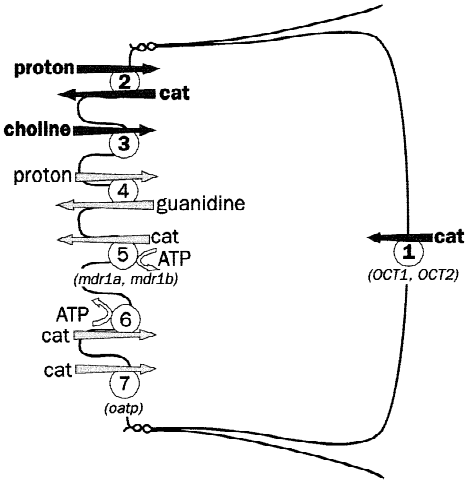

Fig. 1. Organic cation transport systems in plasma membranes of renal proximal tubules. Different transport activities (Nos. 1–6) and an immunologically localized transporter (No. 7) are shown. The best characterized systems are indicated in black. Cloned transporters which probably mediate the respective activities are indicated in parenthesis. In the basolateral membrane a potential dependent, polyspecific organic cation uptake system has been described (No. 1) which mediates the first step in cation secretion. Two homologous transporters that mediate electrogenic transport of small organic cations (*rOCT1* and *rOCT2*) have been localized to the basolateral membrane of rat proximal tubules. The brush-border membrane contains two proton cation antiport activities (Nos. 2, 4) which are responsible for the second step in the secretion of many small organic cations. The secretion of large hydrophobic cations may be mediated by multidrug resistance proteins in the brush-border membrane (No. 5) which are primary active export pumps. The two indicated subtypes of the multidrug resistance protein (*mdr1a, mdr1b*) have been cloned from mouse. Three different uptake systems in the brush-border membrane may be involved in he first step of cation reabsorption. An electrogenic uptake system with preference for choline (No. 3), the polyspecific transporter *oatp* which mediates the translocation for several organic anions and cations (No. 7), and an ATP-dependent cation uptake system (No. 6).

ally distinguished (*see* Fig. 1, *activities* 1–3). Functional studies performed in vivo or with expressed transporters that have been localized to the proximal tubule indicate the existence of four further cation transport systems (Fig. 1, *activities* 4–7). The physiological importance of these systems has not been established. Recent cloning of transporters revealed that polyspecific cation transport systems defined by in vivo measurements may comprise more than one transporter.

Cation Secretion

Cation uptake across the basolateral membrane of tubular epithelial cells is the first step in cation secretion. Measurements with intact tubules and membrane vesicles from the proximal tubule showed that the basolateral uptake of many cations, such as tetraethylammonium (TEA), N^1-methylnicotineamide (NMN), choline, procainamide, cimetidine, amantadine and morphine is mediated by polyspecific uptake systems which are potential dependent but independent of sodium and proton gradients [8, 9, 15, 21–23, 38–41]. The translocated cations are relatively small and may be identical to the substrates of a type 1 transport system in the liver [42–44]. Studies with isolated rat hepatocytes suggested the existence of two uptake systems with different substrate specificities. A type 1 system which translocates small more hydrophilic cations such as procainamide ethobromide and TEA, and a type 2 system which transports larger, more hydrophobic cations such as vecuronium, quinine and d-tubocurarine. The type 1 system was inhibited by type 2 cations but not by taurocholate and *k*-strophantoside, whereas the type 2 system was inhibited by taurocholate and *k*-strophantoside but not by type 1 cations. Similar to the liver, transport of small hydrophilic cations across the basolateral membrane of the proximal tubules was inhibited by type 2 cations [45]. For the inhibition of basolateral cation uptake in proximal tubule by quinine, some stereoselectivity has been demonstrated in rat, rabbit and man [36, 41, 46–48]. Distinct species differences in substrate affinity and different kinetic constants were determined with intact tubules and membrane vesicles. For example, in the rat higher K_m values for TEA uptake were obtained compared with those in the rabbit, and the values determined with membrane vesicles voltage-clamped to zero mV were higher than those determined with intact tubules (rat: intact tubule 0.16 mM [15], vesicles 2.5 mM [38]; rabbit: intact tubule 0.07 mM [8], vesicles 0.37 mM [22]). The different values obtained with intact tubules and vesicles may result from differences in membrane potential (the K_m of the cloned basolateral cation transporter *rOCT1* is potential dependent [32]), from different intracellular concentrations of interacting endogenous substrates or from different regulatory states of the transporter [49]. Basolateral uptake of type 2 cations or cardiac glycosides in the proximal tubule has not been directly demonstrated, however, such transport systems may be postulated since renal secretion of quinine has been observed [48] and proteins that translocate type 2 cations have been identified in the brush border membrane (*see below*). The postulated basolateral type 2 cation transporter in the kidney may also translocate digoxin [50]. It may be identical or homologous to the functionally identified hepatic type 2 cation transporter in sinusoidal membranes [2]. Type 2 cation transporters may belong to the same family as the type 1 cation transporters and renal transporters for organic anions which can both be inhibited by type 2 cations [51]. Following transport across the basolateral membrane organic cations diffuse throughout the cytosol. They may reach the luminal membrane or become sequestered

within intracellular compartments. For example they may be transported by a cation/proton antiporter into acidic intracellular organelles. This cation transporter may be identical to the cation/proton antiporter in the luminal membrane (Fig. 1, *activity* 2). It may be driven by an outwardly directed proton gradient which is generated by an ATP-dependent proton pump [52].

The second transmembrane step in secretion, cation transport over the brush-border membrane, may be mainly performed by the above-mentioned electroneutral cation/proton antiporter (Fig. 1, *activity* 2). In addition a more specific cation/proton antiporter which translocates guanidine (Fig. 1, *activity* 4), and multidrug resistance proteins (Fig. 1, *activity* 5) may be also involved. Polyspecific cation/proton antiport activity has been demonstrated by tracer uptake and *trans*-stimulation experiments with membrane vesicles employing the organic cations TEA, tetramethylammonium (TMA), NMN, choline, mepiperphenidol, procainamide, cimetidine, 1-methyl-4-phenylpyridinium (MPP), and amphoteric aminocephalosporines [8, 9, 18, 21–23, 25, 53–59]. Cross-inhibition experiments showed that this transport activity is mediated by one transport system or by transport systems with closely overlapping specificity. The substrate specificity and affinity for cation/proton antiport across the brush-border membrane was similar to that for basolateral cation uptake. Transport was demonstrated for small organic cations (type 1), whereas larger, more hydrophobic cations were identified as high affinity inhibitors [45, 58, 60]. For TEA/proton antiport activity in brush-border membrane vesicles from rat and rabbit, K_m values of 0.8 and 0.15 mM were estimated [22, 38]. Such systems operate in an electroneutral fashion and are linked to the intracellular pH which is regulated by the sodium/proton exchanger [38, 61, 62]. Studies with right-side out oriented membrane vesicles [63] suggested low substrate specificity and some functional symmetry of the cation/proton antiport. They showed that protons could be replaced by small organic cations. A tight coupling between the translocated organic cations and protons was assumed because during cation uptake in the presence of an initial outwardly directed proton gradient, the intravesicular concentration of transported cations transiently increased over the equilibrium concentration [18, 54, 57, 58]. For cation/proton antiport a stoichiometry of 1:1 was determined [57]. Some structural similarity between cation/proton antiport and anion transport systems within the brush-border membrane was suggested because the classical inhibitor of anion transport, probenecid competitively inhibited cation/proton antiport, albeit with a lower affinity [59]. There may be additional cation/proton antiport systems in the brush-border membrane. For example, in brush-border membranes from rabbit and human proximal tubules and from rabbit intestine guanidine/proton antiport activity has been described which could not be inhibited with TEA and NMN [64–66]. The efflux of large hydrophobic type 2 cations over the brush-border membrane of renal proximal tubules may be small and partially mediated by the drug transporting P-glycoproteins. These are ATP-dependent pumps that mediate the cellular efflux of various drugs and may be responsible for the resistance of cells to antibiotics [67]. In mouse the two transporter subtypes *mdr1a* and *mdrb* have been described [68]. Employing immunohistochemistry and transport measurements P-glycoproteins have been localized to the brush-border membranes of renal proximal tubules [69–73]. Originally the P-glycoproteins were described as ATP-dependent transporters for hydrophobic neutral or anionic drugs, however, later experiments showed that they may be also capable of translocating type 2 cations. For example, after transfection of insect cells with *mdr1b* the cells were capable of translocating the monoquaternary cation *N*-(4′,4′-azo-*n*-pentyl)-21-deoxyajmalinium [74]. Further the cytotoxicity of *Vinca* alkaloids, the typical substrates of P-glycoproteins, was increased in the presence of the cation verapramil [75].

Cation Reabsorption

Bidirectional transport of organic cations in the nephron has been demonstrated. For example, choline is secreted at high plasma concentrations, whereas it is reabsorbed at normal plasma concentrations below 25 μM [10, 39]. At low plasma concentrations the reabsorption may help to prevent the loss of ultrafiltered organic cations, such as choline and monoamine neurotransmitters. In the case of cationic drugs and xenobiotics such reabsorption may underlie nephrotoxicity. The first step in cation reabsorption is the uptake across the brush-border membrane. In the proximal tubule this transport step may be mediated by a potential-dependent transport system (Fig. 1, activity 3 [20, 45, 76]). However, a cation translocating ecto-ATPase which has been described in rabbit renal brush-border membrane vesicles may be also involved. (Fig. 1, *activity* 6 [77]). The multivalent amphiphilic substrate transporter *oatp* which has been localized to the S3 segment of proximal tubules may also participate in the reabsorption of cations (Fig. 1, system 7 [78, 79]). The potential dependent transport system in the luminal membrane was primarily designated as choline transporter because in rabbit the affinity of this system for choline is relatively high [20]. Later experiments in rat suggested polyspecificity of this transporter because it was *cis*-inhibited by a variety of different cations [45]. Significantly different affinities to that for the basolateral cation transporter have been estimated for some cations. For example for TEA inhibition of luminal choline uptake an apparent K_i value of 15.6 mM and for TEA

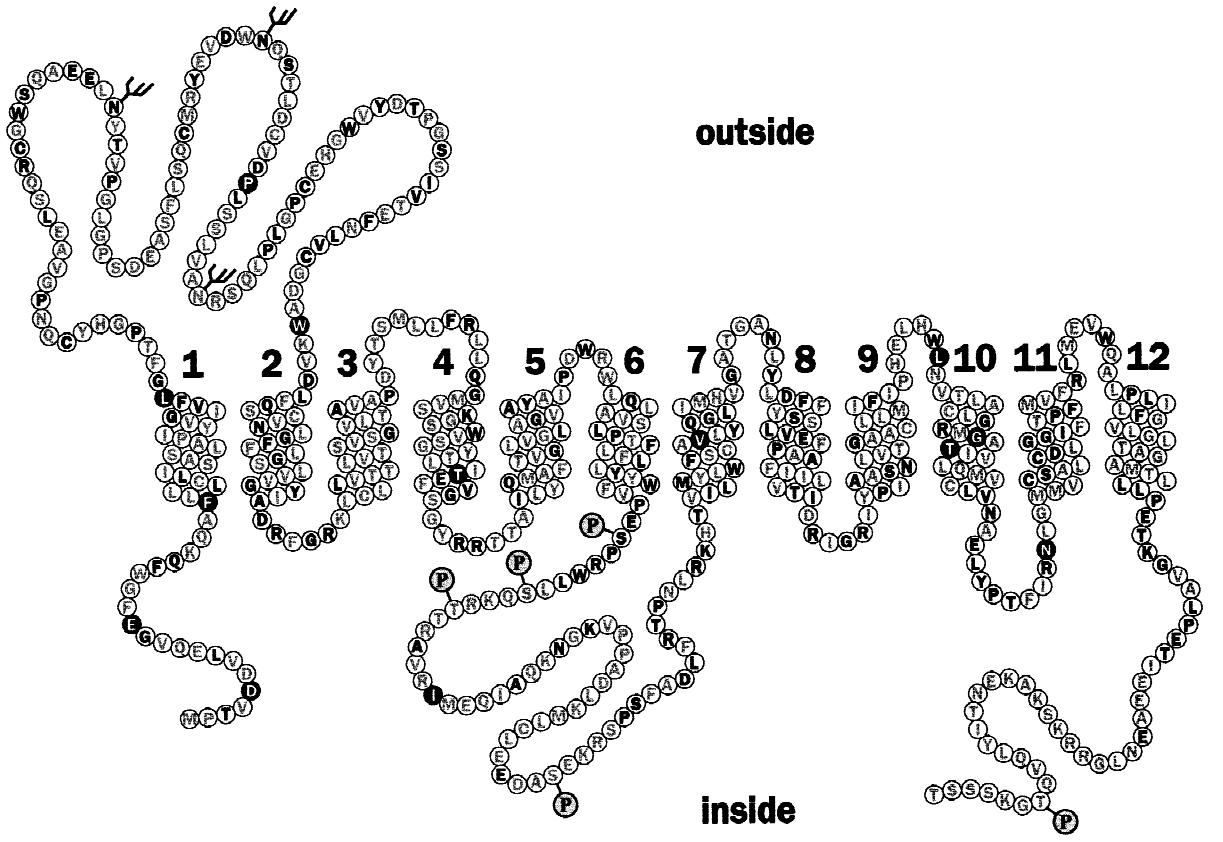

Fig. 2. Amino acid sequence of *rOCT1* and a tentative model of its membrane topology. The amino acids which are conserved between the OCT1/OCT2 subfamily (*rOCT1*, *rOCT2*, hOCT1, hOCT2, pOCT1, *mOCT1*, *mOCT2*, rbOCT1) and rOCT3 are indicated by black letters in bold or by white letters. The white letters, in particular, indicate amino acids which are conserved in all cation transporters (OCT1/OCT2 subfamily plus *rOCT3*, hOCTN1, hOCTN2 and *rOCTN2*) but not in the anion transporters. Consensus sequences for protein kinase C dependent phosphorylation sites (P) and for N-glycosylation sites (ψ) in *rOCT1* are also indicated.

inhibition of basolateral NMN uptake a K_i value of 70 μM was estimated. On the other hand the apparent K_i values determined for *cis*-inhibition of luminal choline and basolateral NMN uptake by 4-(4-dimethylaminostyryl)-N-methyl-pyridinium (4-Di-1-ASP) were 14 μM and 0.28 mM respectively. In brush-border membrane vesicles from rabbit kidney ATP-dependent transport of TEA was demonstrated which was not activated by nonhydrolyzable ATP analogues and could be inhibited by NMN, cimetidine, verapramil and amiloride [77]. This suggests that a primary active, polyspecific cation transporter in the brush-border membrane may also be involved in the first step in organic cation reabsorption. The functional properties and physiological significance of this transporter, which may utilize ATP from the ultrafiltrate, is yet to be elucidated. Since the specificity and activity of *oatp* for cation transport in the S3 segment has not been determined the physiological function of this transporter for cation reabsorption is unclear. The transporters involved in the second step of cation reabsorption, the cellular cation efflux across the basolateral membrane, have not been identified. They may be partially identical to the potential-dependent basolateral cation transporter(s) which are able to transport cations in both directions (*see below*).

Identification of the OCT Transporter Family

Using expression cloning we identified in 1994 a polyspecific organic cation transporter from rat kidney (*rOCT1*) [29] that proved to be the first identified member of a new transporter family. This family was also named SCL22 which stands for solute carrier family 22 [80]. *rOCT1* belongs to the major facilitator superfamily (MFS) which contain twelve transmembrane spanning α-helix motifs and include bacterial drug resistance proteins, proton solute symporters and facilitative diffusion systems for sugars and Krebs cycle intermediates [81]. Figure 2 shows the primary structure of *rOCT1* and a tentative model of its membrane topology. Twelve transmembrane domains are proposed, although hydropathy analysis indicated eleven transmembrane α-he-

Table 1. Cation transport family members

Abbreviation	Chromos.	Organism	Size	Identity to *rOCT1*	Function	Tissue distribution[1]	Reference and accession #
rOCT1	1q11-12	Rat	556	100%	Cation transp.	Liv., kid., intest.	[29,85] X78855
rOCT1A		Rat	430	92%	Cation transp.	Not quant. determined	[34] U76379
rOCT2		Rat	593	67%	Cation transp.	Kid.	[32,33] D83044, X98334
rOCT3		Rat	551	48%	Cation transp.	Plac., intest., heart, brain, kid.	[83] AF055286
mOCT1, Lx1	17	Mouse	556	95%	Not identified	Not determined	[84] U38652, U38913
mOCT2		Mouse	553	69%	Not identified	Not determined	Unpublished, AJ006036
rbOCT1		Rabbit	554	81%	Cation transp.	Liv., kid., intest.	[107] AFO15958
hOCT1	6q25-26	Man	554	78%	Cation transp.	Liv.	[31,35,80] X98332, U77086
hOCT2	6q25-26	Man	555	68%	Cation transp.	Kid., brain	[31,80] X98333
pOCT2		Pig	554	67%	Cation transp.	Not quant. determined	[30] Y09400
hOCTN1		Man	551	33%	Cation transp.	Kid., trachea, b. marrow, etc.	[86] AB007448
hOCTN2	5q31	Man	557	34%	Cation transp.	Heart, plac., s. musc., kid., etc.	[87,88] AF057164
rOCTN2, UST2		Rat	557	33%	Not identified	Not quant. determined	[89] AJ 001933
rOAT1		Rat	551	33%	Anion transp.	Kid., brain	[90,91] AB004559, AF008221
mOAT1, NKT	19	Mouse	546	34%	Not identified	Kid., brain	[93] U52842
flOAT1		Flounder	562	32%	Anion transp.	Not determined	[92] Z97028
rOAT2, NLT		Rat	535	30%	Anion transp.	Liv., kid.	[94,95] L27651
mRST		Mouse	553	30%	Not identified	Kid.	[128] AB005451
UST1		Rat	552	33%	Not identified	Not quant. determined	[89] L27651
drOCT, Orct		Drosophila	548	35%	Not identified	Not determined	[129] Y12400
ceOCT		C. elegans	576	32%	Not identified	Not determined	Unpublished, Z83228

[1] The indicated tissue distribution has been determined by Northern blotting, *in situ* hybridization and/or Western blotting. By polymerase chain reactions with reversely transcribed mRNAs the transcription of *rOCT1, rOCT1A, rOCT2,* pOCT2, hOCT1, hOCT2, rOCTN2 and UST1 could be detected in variety of different localizations ([30,31,34,89] and *own unpublished data*).

lices (TMs 1–3, 5–12). However, in most members of the OCT family TM4 was also predicted. The extracellular localization of the large hydrophilic loop between the first and second predicted transmembrane domain and the intracellular localization of the C-terminus was determined with specific peptide antibodies using permeabilized and nonpermeabilized stably transfected human embryonic kidney (HEK) 293 cells [82]. The extracellular localization of the large hydrophilic loop that contains three potential glycosylation sites of the type NXT/S is also suggested by the demonstration that *rOCT1* is glycosylated (*authors' unpublished data*).

Since 1994 nineteen different gene products with homology to *rOCT1* and a splice variant to *rOCT1* have been identified (Table 1). A sequence alignment of the different gene products is shown in Fig. 3 and an unrooted phylogenetic tree in Fig. 4. Using hybridization techniques the two *rOCT1*-subtypes *rOCT2* and *rOCT3* were identified [32, 33, 83]. *rOCT2* is also expressed in rat kidney and contains 67% identical amino acids to *rOCT1*. *rOCT3* contains 48% identical amino acids to *rOCT1* and is most abundantly expressed in placenta but also in intestine, heart, brain, lung and kidney. Meanwhile homologous cation transporters to OCT1 and OCT2 have been cloned from several species: a splice variant of *rOCT1, rOCT1A* from rat [34], hOCT1 from man [31, 35], hOCT2 from man [31], pOCT2 from pig [30] and rbOCT1 from rabbit [107]. From mouse gene products with high homology to *rOCT1* and *rOCT2* have been cloned but not characterized functionally: *mOCT1* which was first named *Lx1* [84] and *mOCT2* (Genbank accession number AJ006036). The human genes of hOCT1 and hOCT2 (also named SLC22A1 and SLC22A2) have been localized on chromosome 6q26 where they lie in close physical proximity (less than 500 kb) [80]. The genes of *rOCT1* and *mOCT1* (*Lx1*) have been mapped to the syntenic regions on rat chromosome 1q11 → q12 and mouse chromosome 17 respectively [84, 85]. The functional characterization of expressed OCT transporters from different species (*see below*) strongly suggests that the OCT1, OCT2 and OCT3 transporters are polyspecific facilitative diffusion systems for small organic cations which operate in an electrogenic fashion and differ in respect to substrate specificity and localization. In kidneys of rat and man OCT2 is strongly expressed. In kidneys of rat and rabbit also a significant expression of OCT1 was observed. At variance OCT1 was not expressed in the human kidney. Since in all tested species OCT1 was strongly expressed in the liver whereas OCT2 mRNA could not be detected in Northern blots, OCT1 may be the most important electrogenic cation transporter in the liver. Also *rOCT3* may also participate in renal cation transport because in Northern blots of rat kidney *rOCT3*-mRNA could be detected.

Eleven other gene products with twelve putative membrane-spanning α-helices have been identified

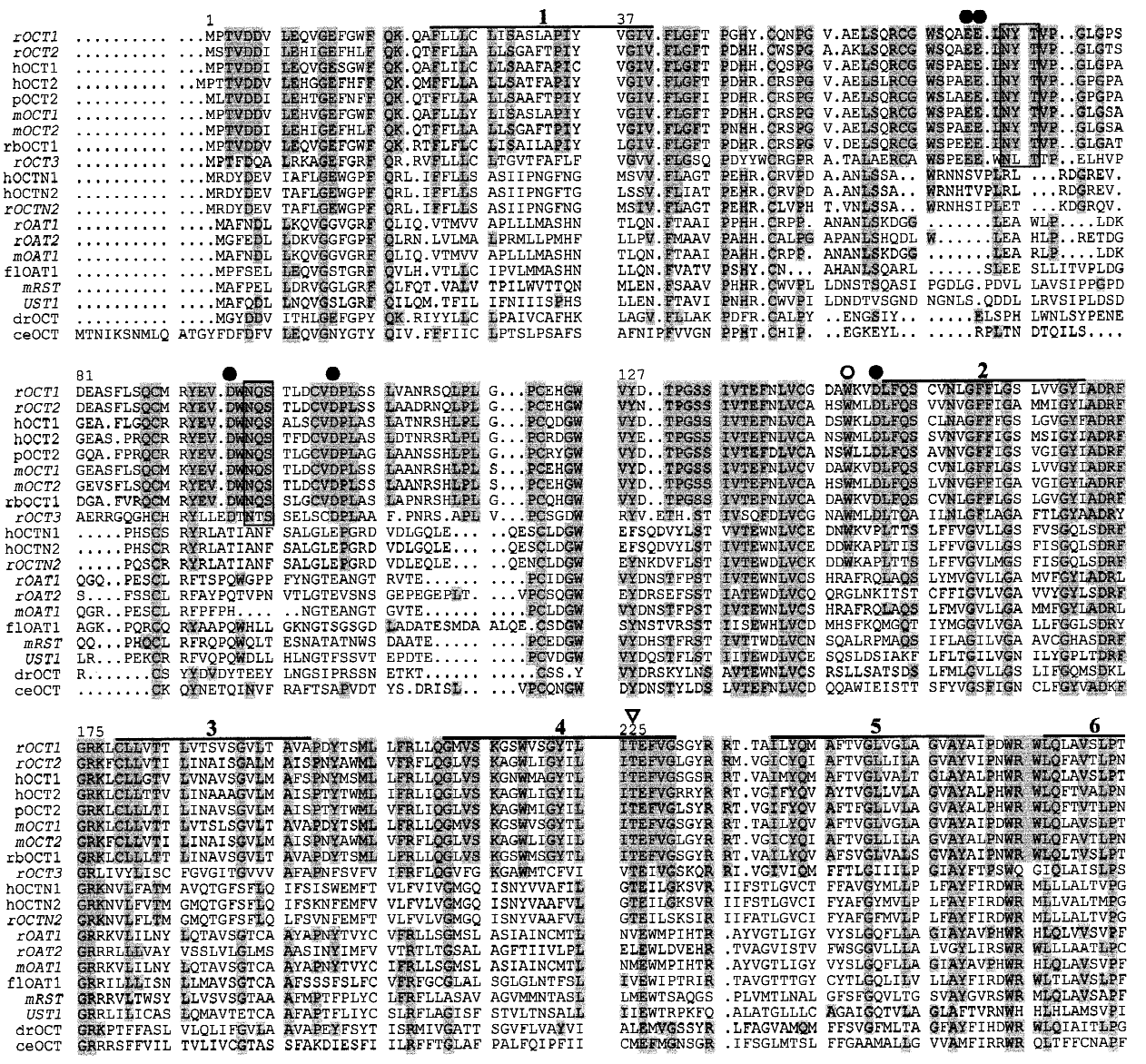

Fig. 3. Sequence alignment of *rOCT1* and the nineteen homologues identified to date. The amino acid residues of *rOCT1* are numbered. The alignment was performed with the GCG programs PileUp and LineUP [130]. The abbreviations for the different genes are described in Table 1. Amino acid residues which are conserved between all sequences of the OCT family or within the OCT1/OCT2-subfamily are shadowed. Acidic amino acids that are conserved in the OCT1/OCT2-subfamily plus *rOCT3* but not in the cation transporters hOCTN1/hOCT2 and *rOCTN2* or the anion transporters *rOAT1, mOAT1, rOAT2* and flOAT1 are indicated by closed circles. Threonine and tryptophane residues which are conserved in all cation transporters but not in the anion transporters are marked by open triangles or an open circle, respectively. Two NXT/S type N-glycosylation sites that are conserved in the OCT1/OCT2 subfamily plus *rOCT3* are boxed. A consensus sequences for protein kinase C-dependent phosphorylation which is conserved between the whole OCT-family is indicated by an asterisk. The predicted membrane-spanning α-helices are overlined.

which reveal between 30 and 35% identical amino acids with *rOCT1* (Table 1, Fig. 4). Transport activities have been determined for hOCTN1, hOCTN2, *rOAT1* and flOAT1. hOCTN1 and hOCTN2 from man were identified by their homology to OCT transporters [86–88]. hOCTN1 is transcribed in kidney, trachea, bone marrow and many other organs. It mediates pH-sensitive transport of TEA and contains a sequence motif for nucleotide binding [86]. A homologous gene product from rat (*UST2*) with 74% identical amino acids also has been isolated [89]. Because *UST2* belongs to the OCT family and has a high homology to hOCTN2 (*see* Fig. 4) it should be renamed *rOCTN2*. hOCTN2 is strongly expressed in heart, placenta, skeletal muscle, kidney, pancreas and more moderately in liver, brain and lung. It may mediate some minor uptake of TEA [87]. However,

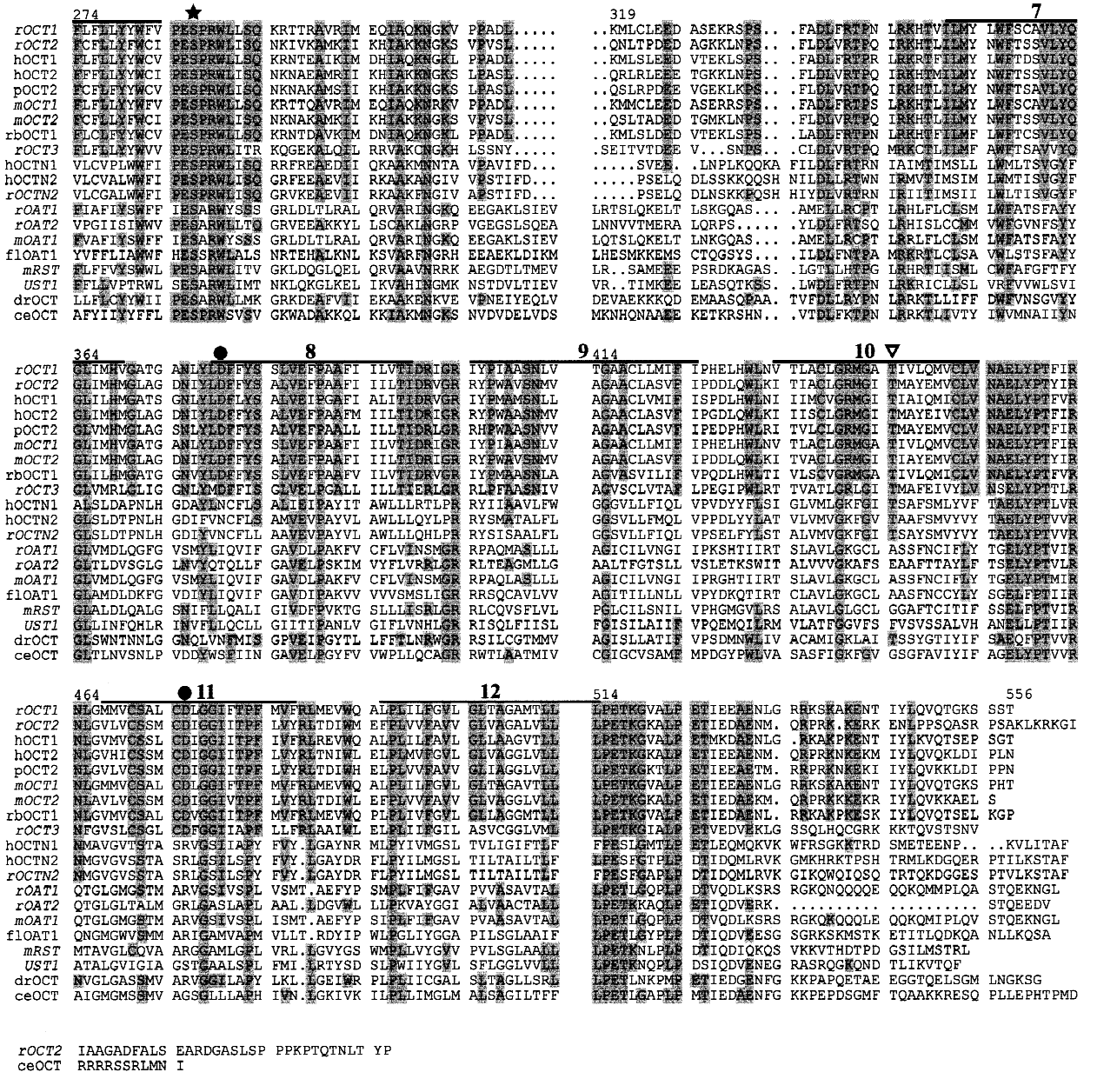

Fig. 3. Continued.

the physiological relevant function of hOCTN2 appears to be sodium-dependent, high affinity transport of carnitine [88].

rOAT1 and flOAT1 are renal polyspecific anion transporters from rat and flounder which have been identified by expression cloning in 1997 [90–92]. In the same year a kidney-specific gene product (*NKT*) has been cloned from mouse that has 95% identical amino acids with the rat anion transporter *rOAT1* [93]. NKT is probably the mouse homologue of this transporter and should be renamed *mOAT1*. Recently it has been shown that the gene product *NLT* from rat which has been already cloned in 1994 but was not functionally identified [94], is another polyspecific anion transporter which may be renamed *rOAT2* [95]. *mRST* from mouse [128], *UST1* from rat [89], drOCT from Drosophila melanogaster [129], and ceOCT from Caenoraphditis elegans (*see* Table 1) are other OCT homologous gene products that have not been functionally characterized. The functional role of the renal proteins *mRST, UST1* and the OCT homologous proteins from drosophila (drOCT) and the nematode Caenorhabditis elegans (ceOCT) cannot be predicted. drOCT and ceOCT may be evolutionary precursors of the mammalian cation and anion transporters.

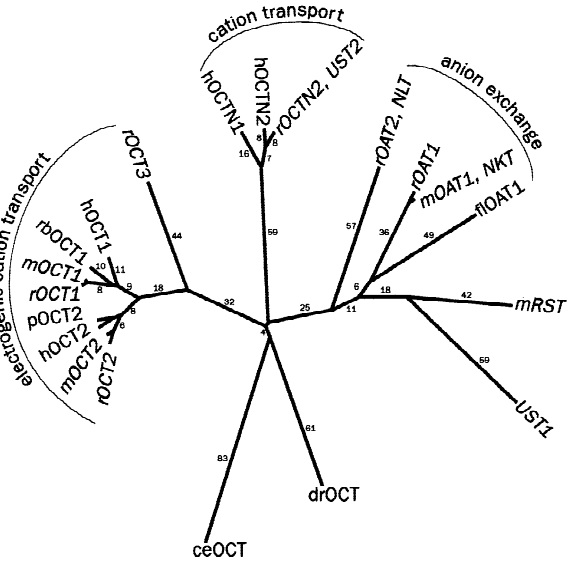

Fig. 4. Unrooted phylogenetic tree of *rOCT1* homologues. The Felsenstein program Drawtree of the PHYLIP package [131] was used to represent the phylogenetic distances in arbitrary units as calculated from the sequence alignment performed as in Fig. 3. Functional activities which have been determined after expression of the respective gene products or in a subtype from another species (*mOCT1, mOCT2, rOCTN1, mOAT1*) are indicated. The electrogenic uptake of small cations over the basolateral membrane of renal proximal tubules (*see* activity 1 in Fig. 1) is mediated by the OCT1/OCT2 subfamily. The localization of *rOCT3, rOCTN1* and *rOCTN2* in the kidney has not been determined.

Conserved Amino Acids in the OCT-Family

In Fig. 3 the amino acid sequences of the 20 OCT family members are compared. Forty-one amino acid residues are conserved between all family members including 7 proline, 7 glycine, 4 cysteine, 4 glutamate and 4 arginine residues. Only 10 of these conserved amino acid residues are localized in the presumed membrane spanning α-helices, and 9 conserved amino acid residues belong to the intracellular transporter signatures which are typical for the major facilitator superfamily [81, 96]. The 4 cysteine residues conserved in the OCT family are localized in the large extracellular loop. This suggests the functional importance of this loop which may contain a disulfide bridge and may protrude into the membrane as has been described for the (Na^+,K^+)ATPase [97, 98]. Inhibition of renal cation and anion transport by sulfhydryl reagents could be due to the reaction with these cysteine residues [99–101]. Figure 4 shows that the OCT1 and OCT2 proteins represent a subfamily to which *rOCT3* is rather homologous. The OCT1/OCT2 subfamily contains 284 conserved amino acid residues and shares very similar functional properties (*see below*). *rOCT3* is closely related to the OCT1/OCT2 subfamily since it shares 196 identical amino acids and also mediates electrogenic cation uptake. Only 91 of these 196 amino acid residues are also conserved in the cation transporters hOCTN1, hOCTN2 and *rOCTN2* which may belong to a less related cation transporter subfamily (*see* Fig. 4). This subfamily may contain sodium-dependent cotransporters [88]. The OCT1/OCT2 subfamily of cation transporters may be identified by the signature **RC**G**WS**X**(A/E)EE**L**NY**T**V**P**G which represents amino acid residues 61 to 76 of *rOCT1*. The amino acids in this signature which are identical to *rOCT3* are printed in bold face. In the OCT1/OCT2 subfamily plus *rOCT3* three more cysteine residues are conserved than in the whole family: one cysteine residue is also localized in the large extracellular loop and two in TM11. The large extracellular loop of *rOCT1* contains three NXT/S-type consensus sequences for N-glycosylation [29]. Two of them may be functional since they are conserved in the OCT1/OCT2 subfamily and in *rOCT3*, and glycosylation of *rOCT1* has been demonstrated (*authors' unpublished data). rOCT1* contains four potential protein kinase C-dependent phosphorylation sites on the large intracellular loop between TM6 and TM7 (Fig. 2). One of these sites is conserved in the whole OCT-family. This potential phosphorylation site may be functional because cation transport expressed by rOCT1 was increased after stimulation of proteinkinase C [102].

The identification of cation bindings sites would represent a first step in the elucidation of the molecular mechanism of cation transport by OCT transporters. Since the involvement of electrostatic interactions is probable, conserved negatively charged, aromatic and polar amino acids within transmembrane α-helices or on extracellular loops may participate in binding domains [103, 104]. Therefore acidic amino acid residues or amino acid residues with negative partial charge that are conserved in the cation transporters but not in the anion transporters should be identified. There are seven acidic amino acids that are conserved in the electrogenic cation transporters of the OCT1/OCT2 subfamily and *rOCT3* but not in the anion transporters and the OCTN1/OCTN2 cation transporter subfamily: two glutamate and three aspartate residues in the large extracellular loop, one aspartate residue in TM 8 and another one in TM 11 (*see* closed circles in Fig. 3). In the cation transporters, hOCTN1, hOCTN2 and *rOCTN2* and in the anion transporters this latter aspartate residue (residue 475 of *rOCT1*) is replaced by an arginine residue. The π-electrons of tryptophan, as well as partial negative charges on serine and threonine residues, may participate in electrostatic cation binding, it is therefore relevant that five threonine, three tryptophan and four serine residues are conserved in the OCT1/OCT2/OCT3 transporter subgroup but not in the anion transporters. Two of the threonine residues (residues 226 and 444 of *rOCT1*) and one of the tryptophan residues (residue 147 of *rOCT1*) are also conserved in hOCTN1, hOCTN2 and *rOCTN2.*

Mutagenesis experiments may elucidate which of the negatively charged or polarized domains on the large extracellular loop and in the transmembrane α-helices are involved in cation binding and transport.

Functional Properties of Expressed Cation Transporters

OCT1 and OCT2 Type Transporters

Cation transport mediated by OCT transporters has been investigated after expression in *Xenopus* oocytes [29, 31–35, 83, 105–107], human embryonic kidney cells [30, 86, 88, 105, 108], HeLa cells [83, 87, 109] and insect cells (*authors' unpublished data*). Tracer uptake experiments in these systems showed that uptake of small organic cations like TEA and 1-methyl-4-phenylpyridinium (MPP) was induced by *rOCT1*, *rOCT1A*, rbOCT1, *rOCT2*, pOCT2, hOCT1 and hOCT2 and could be inhibited by a variety of organic cations with different molecular structures, including the high affinity inhibitors quinine, quinidine, cyanine 863 and decynium 22. The data strongly suggest that OCT1 and OCT2 type transporters from different species operate in a similar fashion: they all appear to be electrogenic facilitative diffusion systems for small organic cations that work independent of sodium and proton gradients. The OCT1 and OCT2 subtypes exhibit differential substrate specificities that are species specific. After expression of OCT1 and OCT2 transporters in *Xenopus* oocytes or human embryonic kidney cells we obtained the same functional characteristics for the initial uptake of cations into both cell types ([105] and *authors' unpublished data*). In the oocytes the expressed cation uptake is linear for 1 hr whereas uptake into transfected HEK 293 cells is only linear for about one second [105, 110]. It is difficult to interpret the measurements that have been performed with monolayers grown on petri dishes [30, 108]. They probably represent transport through the monolayer rather than transport into the cells because *rOCT1* expressed cation uptake measured in such monolayers showed an apparent linearity over a time period of minutes, however, they did for example not exhibit the well documented potential dependence of cation uptake by *rOCT1* ([32, 105] and *unpublished data*).

Some detailed functional analyses have been performed with *rOCT1* [29, 32, 105, 106], *rOCT2* (*own unpublished data*), hOCT1 [31, 35, 109], pOCT2 [30], and hOCT2 [31, 110]. For *rOCT1*, *rOCT2* and *hOCT2* tracer uptake of TEA, MPP, NMN, choline, dopamine, serotonine and histamine was demonstrated. At variance quinine and quinidine were not transported by *rOCT1* or *rOCT2* ([106] and *own unpublished data*). The uptake of TEA and/or MPP by *rOCT1*, *rOCT2*, hOCT1, hOCT2 and pOCT2 was inhibited by the transported cations and by quinine and quinidine. The high affinity inhibitors d-tubocurarine, cyanine 863 and decynium 22 probably also belong to the group of nontransported cations. In general the transported cations are smaller and more hydrophilic. They may be identical to substrates of a type 1 cation transporter which has been described in sinusoidal membranes of rat hepatocytes. This type 1 transporter has been distinguished from a type 2 cation transporter in the same membrane that transports more hydrophobic cations such as quinine and d-tubocurarine [2, 42–44]. For cation transport by OCT1 and OCT2 transporters apparent K_m values between 10 μM (MPP uptake by *rOCT1*) and 600 μM (choline uptake by *rOCT1*) were estimated.

rOCT1, *rOCT2* and hOCT2 expressed in *Xenopus* oocytes have been further characterized using electrophysiological measurements. When oocytes expressing these transporters were superfused with transported cations their membrane potential decreased in contrast to water-injected control oocytes [31, 32, 106, 110]. With voltage-clamped cRNA-injected oocytes positive inward currents were induced after superfusion with transported cations. Currents induced by saturating cation concentrations increased with the membrane potential. We concluded therefore that the expressed cation transport is electrogenic. The cation binding site involved in transport may be localized within the electrical field of the membrane potential because the apparent K_m values for TEA and choline decreased with increasing potential (shown for *rOCT1* [32]).

Preliminary experiments were performed to investigate cation transport by *rOCT1* and hOCT2 for symmetry. If *rOCT1*- or hOCT2-expressing oocytes were preloaded with 1 mM choline the membrane potential increased by 20 to 30 mV, whereas the membrane potential of water-injected control oocytes was not altered. This suggests that the transporters mediate electrogenic efflux of choline or other cations. Efflux of organic cations by *rOCT1* and hOCT2 could be demonstrated directly by measuring the release of [^{3}H]MPP from oocytes and HEK 293 cells [32, 110]. Transporter-mediated MPP efflux was observed even when no cations were in the bath (*trans*-zero). This showed that the OCT1 and OCT2 transporters may function as uniporters. The MPP efflux was inhibited when the bath contained the nontransported cations quinine or quinidine. Such effects should be considered when electrical measurements are interpreted. For example in voltage clamp experiments *trans*-inhibition of electrogenic cation efflux by nontransported cations may be confused with electrogenic cation uptake if the potential dependence is not determined [32, 106]. When transported organic cations such as TEA, choline or MPP were added to the bath of oocytes expressing *rOCT1* or hOCT2 the initial rate of

Table 2. Comparison of the inhibitor sensitivity of ^{14}C labeled uptake of TEA by *rOCT1, rOCT2,* hOCT1 and hOCT2

Inhibitor	Apparent K_i values [μM]			
	rOCT1	*rOCT2*	hOCT1	hOCT2
Cyanine-863	0.71	0.43	–	0.21
Decynium-22	0.36	0.58	2.7	0.10
Tetrapentylammonium	0.53	1.2	7.5	1.5
Desipramine	16	10	5.4	16
Quinine	3.5*	12	23	3.4
Quinidine	12.5	13	18	
Mepiperphenidol	5.2	245		4.8
Procainamide	30	1752	74	50
1-Methyl-4-phenylpyridinium	13	54	12	2.4
O-Methyl-isoprenaline	43	2474		570
Corticosterone	160*	5.8	7.0	29*
Tetramethylammonium	1000	771		180
N-methylnicotinamide	384	255	7700	266

The effect of different inhibitor concentrations on the uptake of ^{14}C TEA expressed in *Xenopus* oocytes by *rOCT1, rOCT2,* hOCT2 was measured and the apparent K_i values calculated. Most values for *rOCT1* and hOCT2 were taken from refs. [29] and [31] whereas our values determined for *rOCT2* have not yet been published. Own unpublished data from *rOCT1* and hOCT2 are indicated by asterisks. The K_i values for hOCT1 were taken from Zhang et al. [109]. These data were obtained with transfected HeLa cells.

[^{3}H]MPP efflux was increased. Recent experiments with hOCT2 showed that this *trans*-stimulation in polarized cells is due to a membrane depolarization by inwardly transported cations. If hOCT2 expressing oocytes were depolarized to −10 mV by replacing sodium in the bath with potassium, the MPP efflux under *trans*-zero conditions was significantly increased. In these depolarized oocytes no *trans*-stimulation of MPP efflux was observed when transported cations were added to the bath which did not induce a significant depolarization under these conditions [110]. To understand the physiological role of OCT1 and OCT2 transporters it has to be known when these transporters may mediate significant cation efflux in vivo. Recent data with depolarized oocytes and HEK 293 cells expressing hOCT2 revealed tenfold higher rates for the influx than for the efflux of 0.1 μM [^{3}H]MPP. Whereas in depolarized cells no *trans*-stimulation could be observed for the efflux of [^{3}H]MPP, for the influx of [^{3}H]MPP a significant *trans*-stimulation by choline and MPP was detected which could not be explained by potential effects of the *trans*-cations [110]. At variance with other transported cations no *trans*-stimulation was detected for the influx of [^{3}H]MPP. This indicates asymmetry of hOCT2. Experiments with macropatches or cut-open oocytes which allow better control of the cation concentrations on both plasma membrane sides are necessary to investigate this topic quantitatively.

To elucidate whether nontransported inhibitors, such as quinine and d-tubocurarine, interact at the same cation binding site of *rOCT1* as the transported organic cations we measured *rOCT1* mediated [^{14}C]TEA influx at different TEA concentrations in the presence of various concentrations of NMN (transported cation) and quinine (nontransported inhibitor). A competitive inhibition was observed for NMN whereas quinine proved to be a noncompetitive inhibitor (*authors' unpublished data*), thus we propose that *rOCT1* contains an allosteric cation binding site. This site may be localized close to the cation transport site but is not protected by transported cations. Recently we also observed that cation transport by *rOCT1* or *rOCT2* can be inhibited by 1 mM of the organic anion probenecid which is a classical high affinity inhibitor of the renal anion transporter [3]. Since the polyspecific organic anion and organic cation transporters belong to the same family *rOCT1* and *rOCT2* may express a type of ''degenerated'' probenecid binding site. The data are consistent with the previous observations that probenecid inhibits cation uptake in renal tissue sections and plasma membrane vesicles [59, 111] and that a variety of substances interact with both contraluminal organic anion and organic cation transport systems in rat proximal tubules [51, 112].

To distinguish OCT1 and OCT2 transporters, apparent K_i values for organic cation uptake inhibitors may be compared (Table 2). The K_i values for transported and nontransported organic cations may reflect organic cation binding affinities of two allosterically interacting cation binding sites, thus when modelling the cation binding sites of these transporters each group of cations should be considered separately. For such a comparison care must be taken that the K_i values of the different transporters are determined under identical conditions; including the experimental setup, the membrane potential, the concentrations of interacting cations and anions, the regulatory state of the transporters, and the presence of interacting proteins [37, 59, 113, 114]. Measurements in vivo and with expressed organic cation transporters differ in many of these parameters. For example in perfused tubules normally transepithelial fluxes are present so that the intracellular substrate concentrations might be lower than in expression systems. Differences in the parameters between measurements in vivo and expressed organic cation transporters may explain why many of the K_i values determined by Ullrich and coworkers [15, 45, 115, 116] for the basolateral cation uptake in rat kidney proximal tubules differ by orders of magnitude from the K_i values that we have determined for expressed *rOCT1* and *rOCT2* transporters localized in the basolateral membrane of rat renal proximal tubules (*see below*). We have determined, for example, K_i values of 0.36 μM and 0.58 μM, for the inhibition of *rOCT1* and *rOCT2* mediated TEA uptake by decynium 22, respectively, whereas Ullrich and coworkers reported a K_i value of 420 μM. For the transported cations TEA, NMN and choline, less

disparate K_i values were determined, however, the K_i values of transported cations may also differ by a factor of 10.

The K_i values for several expressed members of the OCT1/OCT2 subfamily are compared in Table 2. The affinity patterns of the two basolateral rat transporters *rOCT1* and *rOCT2* are different. *rOCT1* has a much higher affinity for some cations (for example mepiperphenidol and procainamide), a similar affinity for others (for example, decynium 22 and quinidine), and a lower affinity for corticosterone. The cation specificity patterns of the human subtypes hOCT1 and hOCT2 are distinctly different from those of *rOCT1* and *rOCT2*. We could not detect a systematic difference between the specificity patterns of the OCT1 and OCT2 subtypes from the human and the rat. At variance to the rat, hOCT1 has a higher affinity for corticosterone than hOCT2, a similar affinity for procainamide, a lower affinity for tetrapentylammonium, and a much lower affinity for NMN. Trying to summarize the species difference between the human and the rat, it may be stated that for several ligands hOCT1 has a lower affinity than *rOCT1* (corticosterone and desipramine are exceptions) whereas hOCT2 often has a higher affinity than *rOCT2*. Stereospecificity for quinine and quinidine was determined for *rOCT1* but not for *rOCT2* and hOCT1. The data indicate distinct differences in the substrate specificities of OCT1- and OCT2-type transporters which are species specific and cannot be predicted from the overall homology of these transporters.

rOCT3

Transport activity by *rOCT3* has been characterized in HeLa cells and *Xenopus* oocytes [83]. It has been shown that *rOCT3* is an electrogenic transporter for TEA and guanidine and is inhibited by a variety of organic cations. For the [^{14}C]TEA uptake mediated by *rOCT3* an apparent K_m value of 2.5 mM was determined. This is about 20-fold higher than the K_m values of *rOCT1* and *rOCT2*. Cation inhibition experiments suggest that *rOCT3* is a polyspecific transporter which has a lower affinity for small organic cations than *rOCT1* and *rOCT2*. It may be able to translocate more bulky organic cations. pH effects on cation transport by *rOCT3* were investigated in detail [83]. It was shown elegantly that an apparent pH dependence of *rOCT3* that was determined by tracer flux measurements could not be confirmed in voltage-clamped oocytes. This is a good example how misinterpretations of pH-effects on transport measurements of electrogenic transporters can be avoided.

hOCTN1 AND hOCTN2

The characterization of cation transport mediated by the OCTN-subfamily is rather incomplete. Transport measurements have been performed with the human subtypes hOCTN1 and hOCTN2 [86–88]. TEA transport by hOCTN1 was analyzed in suspended HEK 293 cells that were transiently transfected with this transporter [86]. At variance to cellular cation uptake by *rOCT1* and hOCT2 which was only linear for seconds [105, 110], TEA uptake by hOCTN1 was linear for one or two minutes. This suggests a different transport mechanism. Since the TEA uptake by hOCTN1 decreased when the outside pH was reduced and when the cells were depleted from ATP it was speculated that hOCTN1 may be identical to the luminal cation/proton antiporter or to the luminal ATP dependent cation transporter. After expression of hOCTN2 in HeLa cells some pH sensitive transport of TEA uptake was detected which could be inhibited by several different cations [87]. While this suggests polyspecific and pH-sensitive cation transport, it was shown recently that hOCTN2 expresses highly active carnitine transport with an apparent K_m value of 4.3 μM [88]. Notably, the uptake of carnitine was sodium-ion dependent. Future experiments will clarify whether hOCTN1 and hOCTN2 are sodium cotransporters or contain sodium activation sites.

Distribution of OCT1 and OCT2 Type Transporters in Kidney

To understand the physiological function of the cloned cation transporters their nephron and plasma membrane localization must be known. Although renal cation transport systems in the basolateral and luminal membranes of proximal tubules have been characterized in great detail, a localization of the cloned transporters on the basis of functional in vivo data remains speculative. *In situ* hybridizations with specific probes, RT-PCR experiments with dissected tubuli and immunohistochemical experiments with specific antibodies are required to localize the cloned transporters. RT-PCR experiments with dissected rat tubuli showed that *rOCT1* and *rOCT2* are transcribed in renal proximal tubules and that *rOCT2* is additionally transcribed in the pars convolutae of distal tubules (*authors' unpublished data*). Employing immunohistochemistry with subtype specific peptide antibodies on well preserved kidney sections we detected *rOCT1* in the basolateral membranes of S1 and S2 segments of renal proximal tubules, whereas *rOCT2* was found in the basolateral membranes of S2 and S3 segments (U. Karbach and H. Koepsell, *unpublished data*). In these experiments *rOCT2* protein was not detected in the distal tubules. The data suggest that both transporters are engaged in the first step in cation secretion in proximal tubules.

There are distinct species differences concerning the tissue distribution and histochemical localization of the

OCT1 and OCT2 transporters. For example *rOCT1* from rat is expressed in kidney and liver, whereas human hOCT1 is only expressed in the liver [29, 31]. The tubular localization and membrane localization of hOCT2 and pOCT2 have not been unequivocally determined. *In situ* hybridization and immunohistochemistry revealed that hOCT2 is mainly expressed in distal convoluted tubules and our immunohistochemical data suggested that hOCT2 is localized at the luminal membrane [31]. To confirm the luminal localization of hOCT2 and pOCT2 further experiments are required because the luminal localization of hOCT2 was determined with human kidney sections of relatively poor quality, the detection of weak hybridization and immunohistochemical signals was therefore not possible. Gründemann and coworkers [30] compared the affinities of several inhibitors on the luminal TEA uptake into monolayers of LLCPK1 cells and of pOCT2 expressing HEK 293 cells. Their interpretation that pOCT2 represents the luminal cation transporter of the LLCPK1 cells is not convincing because the apparent K_i values of decynium 22 and cyanine 863 differed by factors of 8 and 5 respectively, the functional characterization of pOCT2 was incomplete and ambiguous, and immunohistochemistry with specific antibodies was not performed.

Conclusions

With the identification of the OCT family a new age in the elucidation of renal cation excretion and reabsorption has begun. The available data suggest that the OCT family contains subfamilies of different polyspecific transporters that may translocate cations and anions. The OCT1/OCT2 subfamily contains polyspecific facilitated diffusion systems for small organic cations which operate in a rheogenic fashion. There are two or more other subfamilies of organic cation transporters. One subfamily may be related to *rOCT3*. It may also mediate polyspecific and electrogenic cation transport but may show a different membrane localization and substrate specificity as the OCT1/OCT2 subfamily. A third subfamily includes the OCTN1/OCTN2 transporters which may be high affinity sodium cotransporters of zwitterionic solutes. The polyspecific proton cation antiporter at the luminal brush-border membrane which mediates the second step in renal cation secretion does probably not belong to the OCT family. A recent report suggests that this transporter is identical to *ORCTL2* which is homologous to drug efflux pumps in bacteria. *ORCT2* was localized to the brush border membrane of rat renal proximal tubules and it was shown to confer chloroquine and quinidine resistance to bacteria [117].

To understand cation transport in the kidney all members of renal cation transporters must be identified, localized and functionally characterized. Thus it has to be determined whether they operate in the luminal or basolateral plasma membranes and how their activity and membrane distribution is regulated. The functional characterization should be performed under conditions of controlled buffer composition on both plasma membrane sides and the transporters should be investigated for symmetry and electrical properties. Factors responsible for different cation affinities measured in intact tubules and after expression of the cloned transporters are yet to be determined. Because polyspecific transporters for cations, anions and noncharged solutes have overlapping substrate specificities, the different types of polyspecific transporters in the kidney must be considered when the interaction of drugs and their renal toxicity is discussed. For example it is known from clinical studies that the renal excretion of digoxin is inhibited by quinidine [118, 119]. To date three polyspecific transporters have been identified in the kidney which translocate or bind digoxin and interact with quinidine: P-glycoproteins (MDR), the polyspecific anion transporter *oatp*, and the polyspecific cation transporters OCT1/OCT2; however, our knowledge on the human transporters is not sufficient to explain the clinical observations [120–127].

It is a demanding challenge for the future to elucidate the molecular mechanisms of polyspecific electrogenic cation transport and polyspecific cation/proton antiport. Therefore these proteins must be crystallized, their tertiary structure determined by X-ray analysis and the cation binding sites characterized by affinity labeling and site-directed mutagenesis. Because organic cation transporters exhibit marked species differences it is of high biomedical importance to identify, clone, characterize and localize all the human cation transporters. Expression systems with these transporters may identify new drugs with optimized secretion properties. Knowledge regarding the structure of cation binding sites and transport mechanisms may help to design such drugs and to modulate transporter activities.

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Note Added in Proof

Recently a paper by Gründemann and coworkers was published (*Nature neurosc.* **1:**349–352) where cloning of a gene with 89% amino acid identity to *rOCT3* and 57% amino acid identity to hOCT1 was reported which was localized very close to the gene locus of hOCT1 and hOCT2 on chromosome 6. Based on the findings (i) that this gene is also transcribed in brain, (ii) that the gene product mediates cation uptake with a similar substrate specificity as cation uptake in Caki-1 cells, and (iii) that the uptake in Caki-1 cells has a similar specificity as the

previously functionally defined ''noradrenaline uptake 2'' system the gene was named EMT which stands for extraneuronal monoamine transporter. Since this nomenclature does not consider the relation to the OCT family and the close homology to the earlier reported *rOCT3* gene we recommend to rename the gene hOCT3.

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