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Expert Review

Polyspecific Organic Cation Transporters: Structure, Function, Physiological Roles, and Biopharmaceutical Implications

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Abstract. The body is equipped with broad-specificity transporters for the excretion and distribution of endogeneous organic cations and for the uptake, elimination and distribution of cationic drugs, toxins and environmental waste products. This group of transporters consists of the electrogenic cation transporters OCT1-3 (*SLC22A1-3*), the cation and carnitine transporters OCTN1 (*SLC22A4*), OCTN2 (*SLC22A5*) and OCT6 (*SLC22A16*), and the proton/cation antiporters MATE1, MATE2-K and MATE2-B. The transporters show broadly overlapping sites of expression in many tissues such as small intestine, liver, kidney, heart, skeletal muscle, placenta, lung, brain, cells of the immune system, and tumors. In epithelial cells they may be located in the basolateral or luminal membranes. Transcellular cation movement in small intestine, kidney and liver is mediated by the combined action of electrogenic OCT-type uptake systems and MATE-type efflux transporters that operate as cation/proton antiporters. Recent data showed that OCT-type transporters participate in the regulation of extracellular concentrations of neurotransmitters in brain, mediate the release of acetylcholine in non-neuronal cholinergic reactions, and are critically involved in the regulation of histamine release from basophils. The recent identification of polymorphisms in human OCTs and OCTNs allows the identification of patients with an increased risk for adverse drug reactions. Transport studies with expressed OCTs will help to optimize pharmacokinetics during development of new drugs.

KEY WORDS: drug transporters; MATE1; OCT1; OCT2; OCT3; OCT6; OCTN1; OCTN2; organic cation transport; polyspecific transporters.

INTRODUCTION

Until recently, the role of transporters during reabsorption and excretion of drugs received only minor attention. This changed when a variety of ATP-dependent polyspecific efflux transporters of the ATP binding cassette (*ABC*) family (1) and polyspecific uptake transporters from four families of ATP independent transporters were identified. These families are the H^+/oligopeptide cotransporter family *SLC15* (2), the organic anion transporting family *SLC01* (3,4), the organic cation/anion/zwitterion transporter family *SLC22* (5,6), and the multidrug and toxin extrusion (MATE) H^+/drug antiporters (7). Whereas most plasma membrane transporters are "oligospecific," i.e. specialized for translocation of specific metabolic or nutritional compounds, "polyspecific" transporters accept compounds with different sizes and molecular structures. These transporters may exhibit large variations in affinity and turnover for different compounds and may have specific physiological roles. In addition, they serve to transfer many drugs across plasma membranes and are involved in drug uptake in small intestine and in drug excretion in liver and kidney.

This review summarizes our current knowledge about structure, distribution, specificity, physiological roles, biopharmaceutical roles and transport mechanism of polyspecific organic cation transporters. Polyspecific organic cation transporters belong to the *SLC22* family and the MATE family. The *SLC22* family contains the three subtypes of passive diffusion organic cation transporters called OCT1 (*SLC22A1*), OCT2 (*SLC22A2*) and OCT3 (*SLC22A3*), the cation and carnitine transporter OCTN1 (*SLC22A4*) that may be a proton cation exchanger, the Na^+-carnitine cotransporter OCTN2 (*SLC22A5*) that can also operate as Na^+ independent transporter for organic cations, and the carnitine and cation transporter OCT6 (*SLC22A16*). These transporters have been reviewed earlier (5,6,8–11), however, recently new data were obtained that provided new insights into physiological functions, biomedical importance and structure-function relationships. For example new localizations of OCT-transporters were detected, the substrate specificity of the OCTs and OCTNs was further defined, and unexpected physiological functions and additional pathophysiological roles of OCTs and OCTNs were identified. In addition, mutagenesis experiments and modeling increased our understanding how OCTs can bind and translocate com-

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pounds of diverse molecular structures. Finally, proton cation antiporters of the MATE family have been identified that participate in the excretion of organic cations (7).

CLONING OF POLYSPECIFIC ORGANIC CATION TRANSPORTERS

OCT und OCTN Transporters

The *SLC22* family is a member of the major facilitator superfamily MFS that comprises transporters from bacteria, plants, animals and humans in 18 transporter families (12). The first transporter of the *SLC22* family in mammals, the rat organic cation transporter OCT1 (*SLC22A1*), was cloned in 1994 (13). Later 16 additional human family members and many orthologs from different species were identified (6). In addition to four organic anion transporters OAT1-4 (*SLC22A6,7,8,11*) and one urate transporter URAT1 (*SLC22A12*), the *SLC22* family includes three organic cation transporters (hOCT1-3 or *SLC22A1-3*) and three transporters for carnitine and/or cations (OCTN1 or *SLC22A4*, hOCTN2 or *SLC22A5*, hCT2 or OCT6 or *SLC22A16*) (phylogenetic tree see Fig. 1 in (5)). Like most members of the *SLC22* family, the organic cation and carnitine transporters have a predicted membrane topology that comprises 12 α-helical transmembrane domains (TMDs), an intracellular N-terminus, a large glycosylated extracellular loop between TMDs 1 and 2, a large intracellular loop with phosphorylation sites between TMDs 6 and 7, and an intracellular C-terminus. OCT1 has been cloned from human, rat, mouse and rabbit (13–17), OCT2 from human (14,15), rat (18), mouse (19), rabbit (16) and pig (20) and OCT3 from human, rat and mouse (21–23). A functional active isoform of rat OCT1 (rOCT1A) missing the first TMDs and the large extracellular loop (24) and a functional active isoform of human OCT2 (hOCT2B) lacking the last three TMDs have been cloned (25). In human the genes coding for OCT1, OCT2 and OCT3 are localized within a cluster on chromosome 6.q26-7 (21,26,27). Each of the three genes comprises 11 exons and 10 introns (27–29). OCTN1 and OCTN2 have been cloned from human, rat, mouse (30–35), and OCTN3 from mouse (32). In human the genes coding for OCTN1 and OCTN2 are localized in a cluster on chromosome 5q31 (33,36). hCT2 also called hOCT6 (gene product of *SLC22A16*) is another carnitine and cation transporter with 12 predicted TMDs that has been cloned from human (37,38). This gene is located on chromosome 6q21-22.1.

MATE Transporters

Recently three mammalian proton cation antiporters called MATE1, MATE2-K, and MATE2-B have been cloned. They belong to the multidrug and toxic compound extrusion (MATE) protein family that is distributed in eukaryota, archaea and eubacteria. MATE1 has been cloned from human, mouse and rat (7,39–41). From human three MATE subtypes have been cloned named MATE1, MATE2-B and MATE2-K (7,42) but so far proton organic cation antiport has only been demonstrated for MATE2-K. A MATE2 protein has been also cloned from mouse (7). For MATE1 and MATE2 a membrane topology of 12 α-helical TMDs has been predicted. In humans the genes encoding MATE1 and MATE2 are located on chromosome 17p11.2 (7).

Tissue Distribution and Membrane Localization

OCT1 (SLC22A1). The polyspecific cation transporter OCT1 exhibits a broad tissue distribution (Table I). OCT1 is expressed in epithelial cells and in some neurons (6,13,43–50). In human it is most strongly expressed in the liver (15) whereas in rodents, OCT1 is strongly expressed in liver,

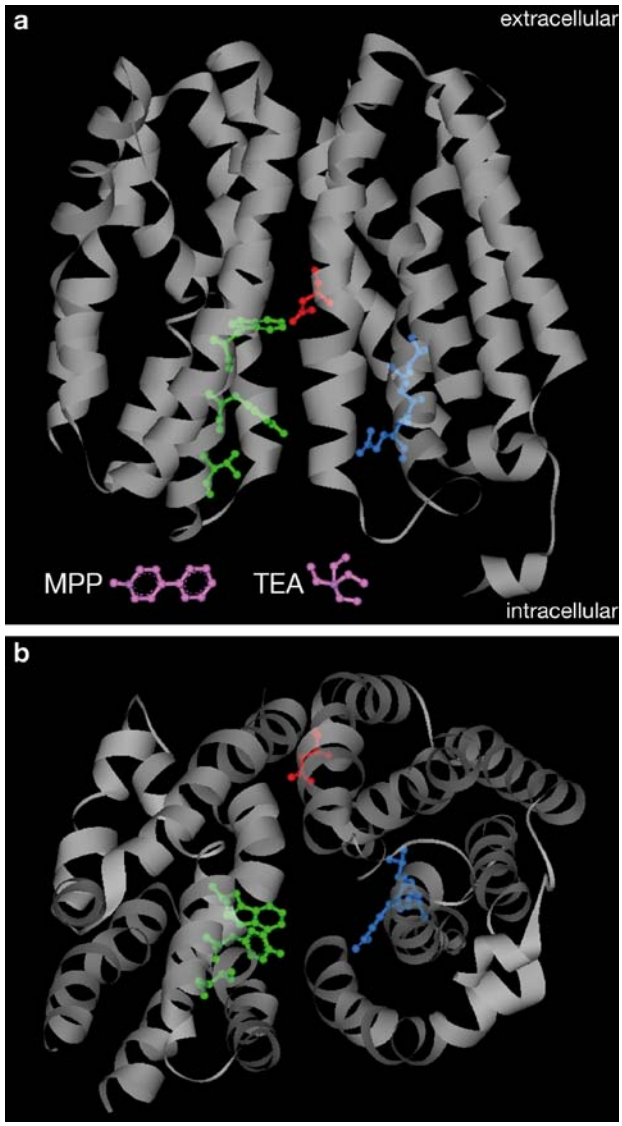

Fig. 1. Structure model of rOCT1 using the tertiary structure of lactose permease (LacY) from *E. coli*. Ribbon representations of the structural model of rOCT1 are presented; (**a**) side view, (**b**) view from intracellular. The model was calculated using the crystal structure of LacY. The molecular structures of TEA and MPP are indicated at the same scale. Amino acids that have been localized to the substrate binding region are depicted. Tryptophan 218, tyrosine 222, and threonine 226 on the fourth transmembrane domain (TMD) are colored in *green*, alanine 443, leucine 447, and glutamine 448 on the tenth TMD are *blue*, and aspartate 475 on the 11th TMD is shown in *red*.

Table I. Tissue Distribution of Polyspecific Organic Cation and Carnitine Transporters[a,b,c]

	OCT1		OCT2		OCT3		OCTN1		OCTN2		OCTN3	OCT6	MATE1		MATE2-K	
	hu	rod	hu	rod	hu	rod	hu	rod	hu	rod	rod	hu	hu	rod	hu	rod
Stomach	+	+	−	−	−	+	∅	+	+	+	∅	−	−	+	−	−
Small intestine	+	**+**	+	+	+	+	+	+	+	**+**	+	∅	−	+	−	−
Large intestine	+	+	∅	−	∅	+	+	+	+	+	∅	∅	−	−	−	−
Rectum	−	−	−	−	−	−	∅	−	+	−	−	−	−	+	−	−
Liver	**+**	**+**	∅	**+**	**+**[c]	+	+	+	**+**	+	−	∅	**+**	**+**	−	−
Pancreas	−	−	−	−	−	−	−	−	+	−	−	∅	−	+	−	−
Spleen	+	+	+	−	∅	+	+	+	∅	+	−	∅	−	+	−	−
Trachea	+	+	+	+	+	+	**+**	−	+	−	−	−	−	−	−	−
Lung	+	+	+	+	+	+	+	+	+	+	+	∅	−	+	−	−
Kidney	+[c]	**+**	**+**	**+**	+[c]	+	**+**	**+**	**+**	**+**	∅	∅	**+**	**+**	**+**	−
Urinary bladder	+	+	∅	∅	+	+	−	+	−	+	−	−	−	+	−	−
Prostate	−	+	−	−	−	+	+	+	+	+	−	∅	−	+	−	−
Testis	−	+	−	∅	−	+	+	+	+	+	**+**	**+**	−	+	−	+
Sertoli cells	−	+	−	∅	−	+	−	+	+	+	−	−	−	−	−	−
Sperm	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
Skin	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	−	+	−	+	−	−	−	−	−	−
Skeletal muscle	+	+	∅	∅	**+**	+	**+**	+	**+**	+	−	∅	**+**	+		—
Heart	+	+	−	∅	**+**	**+**	−	+	**+**	+	∅	∅	+	**+**	−	−
Blood vessels	−	+	−	∅	+	+	−	−	−	+	−	−	−	+	−	−
Brain	+	+	+	+	+	+	+	+	+	+	∅	∅	−	+	−	−
Spinal cord	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
Choroid plexus	−	+	−	+	−	+	−	+	−	+	−	−	−	−	−	−
Adrenal gland	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
Mammary gland	+	+	∅	∅	+	+	+	−	+	−	−	−	−	−	−	−
Uterus	−	∅	−	∅	−	**+**	−	+	+	+	+	−	−	−	−	−
Placenta	+	∅	+	∅	**+**	**+**	+	+	**+**	+	+	∅	∅	+	−	−
Ovary	−	∅	−	∅	−	**+**	−	+	−	+	+	∅	−	−	−	−
Thymus	−	+	−	+	∅	+	+	+	+	∅	−	∅	−	−	−	−
Bone marrow	−	−	−	−	−	−	**+**	−	∅	−	−	+	−	−	−	−
Epithelial cells	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−
Neurons	−	−	+	+	+	+	−	−	−	−	+	−	−	−	−	−
Glial cells	−	−	−	−	+	−	−	∅	−	+	∅	−	−	+	−	−
Muscle cells	−	−	∅	∅	+	+	+	+	+	+	−	−	−	+	−	−
Granulocytes	−	+	−	−	−	+	+	−	+	−	−	−	−	−	−	−
Lymphocytes	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
Macrophages	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
Tumor cells	+	−	+	−	+	−	−	−	+	−	−	**+**	−	−	−	−

[a] The expression was demonstrated by Northern blots, RT–PCR, Western blots or immunohistochemistry. + bold face indicates very strong expression, ∅ no expression detected, −expression has not been investigated.
[b] Lips, K.S., Zapf, F., Volk, C., Müller, J., Brandsch, M., and Koepsell, H., unpublished data.
[c] Lips, K.S., Kummer, W., Ciarimboli, G., Schlatter, E., and Koepsell, H., unpublished data.

kidney and small intestine. In human and rodents OCT1 was also detected in many other organs and/or in tumor cells and basophilic granulocytes (Table I, (14,15,46–48,50–59)). In human and rat liver OCT1 was located to the sinusoidal membrane of the hepatocytes ((60), and unpublished data of Lips K.S., Kummer, W., Ciarimboli G., Schlatter E., and Koepsell H.). In mouse small intestine OCT1 was located to the basolateral membrane of enterocytes (43), and in rat kidney OCT1 was located to the basolateral membrane of epithelial cells in the S1 and S2 segments of proximal tubules (45,61). At variance, in the trachea and bronchi of human, rat and mouse OCT1 is located in the luminal membrane of epithelial cells (47,62).

OCT2 (SLC22A2). The polyspecific cation transporter OCT2 has a more restricted expression pattern than OCT1 or OCT3 (Table I). Similar to OCT1, OCT2 is expressed in epithelial cells and neurons. OCT2 is most strongly expressed in the kidney (15,18) but also in a variety of other organs including small intestine, lung, skin, brain and choroid plexus (Table I, (15,38,46–49,55–59,63,64)). In human kidney OCT2 is expressed in all three segments of proximal tubules (65) whereas in rat kidney OCT2 was located to the S2 and S3 segments (45,61). Similar to OCT1, OCT2 was localized to the basolateral membrane of epithelial cells in renal proximal tubules and small intestine and to the luminal membrane of epithelial cells in trachea and bronchi (45,47,61,65). In rat brain OCT2 was localized to the apical (ventricular) membrane in epithelial cells of choriod plexus (64).

OCT3 (SLC22A3). The tissue expression pattern of the polyspecific cation transporter OCT3 is very broad. At variance to OCT1 and OCT2, OCT3 is not only expressed in epithelial cells and neurons but also in muscle cells and glial cells (50,62,66–70). In human the strongest expression was found in skeletal muscle, liver, placenta and heart, however,

OCT3 was also expressed in many other organs including brain and in some cancer cell lines (Table I, (21,23,50–52,71,72)). In rodents, expression of OCT3 was detected in additional organs, Sertoli cells, and basophile granulocytes (48,49,57–59,64,70). OCT3 protein was localized to the basolateral membrane of the trophoblast in placenta (71), to the sinusoidal membrane of hepatocytes, and to the basolateral membrane of epithelial cells of renal proximal tubules (unpublished data of Lips K.S., Kummer W., Ciarimboli G., Schlatter E., and Koepsell H.). At variance, OCT3 protein was localized to luminal membranes of bronchial epithelial cells and small intestinal enterocytes (47,50)). In brain of rodents OCT3 was observed in hippocampus, area postrema, subfornical organ, medial hypothalamus, and ependym of the third ventricle (44,73,74).

OCTN1 (SLC22A4). The cation and carnitine transporter OCTN1 is expressed in epithelial and muscle cells of various tissues. In human the strongest expression was observed in kidney, skeletal muscle, bone marrow, and trachea (31,75). Weaker expression of human OCTN1 was observed in many other organs (Table I, (31,36,51,76,77)). In rodents compared to humans expression of OCTN1 was observed in additional organs and in Sertoli cells (Table I, (30,46–49,59,78)). In bone marrow OCTN1 mRNA was detected in $CD68^+$ macrophages, $CD43^+$T cells and $CD14^+$ mononuclear cells (36,75). Mouse OCTN1 has been localized to the apical membrane of cortical proximal tubular epithelial cells (79). Recently it has been reported that human OCTN1 is also located in mitochondria (77).

OCTN2 (SLC22A5). The carnitine and cation transporter OCTN2 has a relatively ubiquitous distribution and is expressed in epithelial cells, muscle cells, glial cells, macrophages, lymphocytes and sperm (33,36,51,76,80–82). In humans the strongest expression of hOCTN2 was observed in liver, kidney, skeletal muscle, heart and placenta (Table I, (33,51,80–82). Expression of OCTN2 was also detected in some cancer cell lines (38). In rodents OCTN2 was detected in additional organs, blood vessels, and astrocytes (46,48,49,59,82). In kidney of rat and mouse OCTN2 protein has been localized to the apical membrane of proximal tubular epithelial cells (83). In the epididymal duct of mice OCTN2 was localized to the apical membrane of principal cells (84).

OCTN3 (Slc22a9). This carnitine transporter has been cloned from mice and rat where it is mainly expressed in

Table II. Endogeneous Substrates and Inhibitors of Polyspecific Organic Cation Transporters

Class	K_m or (IC_{50}) [μM][a, b]								References
	hOCT1	hOCT2	hOCT3	hOCTN1	hOCTN2	hOCT6	hMATE1	hMATE2-K	
Metabolites									
Acetyl-L-carnitine					**8.5**				(99)
Betaine					(<500)				(100,101)
Choline	(16,700)[c]	**210** (**381**)	(23,800)[c]	(231)	(**<2,000**)		(>10,000)		(7,15,36, 100–103)
Creatinine	(>20,000)	(**<2,000**)[c]	(15,700)[c]					substrate	(42)
D-Carnitine				(<2,000)	**11, 98**	(30)			(37,99, 101,102)
L-Carnitine	(12,400)[c]	(13,000)[c]	(5,590)[c]	(**24**)	**4.3, 4.8**	**20**			(36,37,99,101,102)
Guanidine	(**5,030**)[c]	(**2,300**)[c]	(1,300,6,200)[c]			(<12,000)	(>2,000)	substrate	(23,42,87, 102–104)
Thiamine								substrate	(42)
Neurotransmitters									
Acetylcholine	(**580**)	**117** (**149**)	(10,490)[c]						(47,105)
Dopamine	(>20,000)[c]	**390–1,400**	1,200[c]			(<12,000)			(63,87,105)
Epinephrine	(>30,000)[c]	**400**	**240**						(105)
Histamine	(>20,000)[c]	**940, 13,00**	**180, 220**						(63,103–105)
Norepinephrine	(7,100)[c]	**1,500, 1,900**	**510–2,600**						(21,63,105)
Serotonin	(>20,000)[c]	**80, 290** (**310**)	(1,000)[c]			(<12,000)	(>200)		(7,87,103,105)
Hormones									
Aldosterone					(<500)				(99)
Corticosterone	(7, 22)	(34)	(0.12, 0.29)		(<100)		(<50)		(7,21,52,99, 107)
Progesterone	(3.1)	(27)	(4.3)				(<50)		(7,52)
Prostaglandin E_2	0.66	0.03							(52,95,96)
Prostaglandin $F_{2\alpha}$	0.48	0.33							(95,96)
Testosterone	(10)[c]	(3)[c]	(44)[c]				(<10)		(7)
Miscellaneous									
Agmatine	(**24,000**)	**1,400** (**3,251**)	**2,500**						(106)
Ergothioneine[d]				**21**					(108)
Stachydrine[d]				**270**					(108)

[a] K_m and IC_{50} values were measured in oocytes of *Xenopus laevis* or epithelial cell lines after expression of the transporters.
[b] Bold face indicates cations for which transport has been demonstrated.
[c] Koepsell et al., unpublished data.
[d] Compounds in the blood that are normally taken up with the food.

testis. Expression of OCTN3 has been also detected in other organs and neurons (Table I (32,49,85)). In small intestine of rat OCTN3 has been assigned to the basolateral membrane of the enterocytes (85). Since antibodies against mouse OCTN3 (mOCTN3) that did not bind to hOCTN1 and OCTN2 from mouse but stained a band of appropriate size in Western blots in human tissue, the existence of a human not yet identified OCTN3 gene has been postulated (86). The same antibody stained a protein that is located in human peroxisomes.

OCT6 or CT2 (SL22A16). Human OCT6 is mainly expressed in testis (37,38). In addition, OCT6 was detected in embryonic liver, in hematopoietic cells, in leukemias and in some cancer cell lines (38,87). In testis OCT6 is located in plasma membranes of Sertoli cells and in the luminal membrane of epithelial cells in the epididymus (37).

MATE1. In humans the MATE1 transporter is strongly expressed in liver, kidney and skeletal muscle and was also detected in heart. (7) Species differences in expression were observed between human and rodents as well as between mouse and rat. In mice strong expression of MATE1 was observed in kidney, liver and heart whereas in rats strong expression of MATE1 was only detected in kidney. (7,39,40) In both rat and mouse some expression of MATE1 was also observed in stomach, spleen, lung, prostate, skeletal muscle, and brain (39–41). At variance to mouse, no expression of MATE1 was detected in small intestine, heart, liver and testis of rat. The additional localizations of MATE1 in rodents described in Table I have been only tested for mice (39). In this study MATE1 was also detected in endocrine pancreas, thyroid gland, parathyroid gland, Leydig cells, and capillaries. In mouse kidney MATE1 was localized to the brush border membrane of renal proximal tubules (7) whereas in mouse liver MATE1 was detected in biliary membranes of hepatocytes, in bile duct epithelial cells, and in Ito cells (7,39).

MATE2. MATE2-K is predominantly expressed in human kidney where it is located in the brush-border membrane of renal proximal tubules. At variance, expression of human MATE2-B has been detected in many organs but not in the kidney (42). In Northern blots mouse MATE2 has been only detected in testis (7).

FUNCTIONAL PROPERTIES AND SUBSTRATE SPECIFICITIES

Common Functional Properties of OCT1-3

The basic transport characteristics of OCT1, OCT2 and OCT3 are similar in various species. First, OCT1-3 translocate a variety of organic cations with widely differing molecular structures, and are inhibited by a large number of additional compounds that are not transported (Tables II, III, IV and V). The relative molecular mass of most compounds that are transported by OCT1-3 is below 500 and the smallest diameter of the molecules is below 4 Å (88). Second, OCTs translocate organic cations in an electrogenic manner. Electrogenicity of transport has been shown for the rat transporters rOCT1, rOCT2, and rOCT3 (13,22,89–92), and for the human transporters hOCT1 and hOCT2 (15,63,93). Third, OCTs operate independently of Na^+, and are independent of proton gradients when the effect of proton gradients on the membrane potential is excluded (15,22,92,94). Fourth, OCTs are able to translocate organic cations across the plasma membrane in either direction. In addition to cation influx, cation efflux has been demonstrated for rOCT1, rOCT2, hOCT2, rOCT3 and hOCT3 (22,47,63, 91,92).

Most substrates translocated by the OCT transporters are organic cations and weak bases that are positively charged at physiological pH, but non-charged compounds (for example cimetidine at alkaline pH) may be also transported (Tables II, III, IV and V). So far, the observation of Kimura et al. (95) that the anions prostaglandin E_2 and $F_{2\alpha}$ are transported by hOCT1 and hOCT2 could not be repeated ((96), and unpublished data of Koepsell H.). Transported substrates of the OCT-transporters are endogenous compounds (Table II), drugs (Table III), xenobiotics, and model compounds (Table IV). 1-Methyl-4-phenylpyridinium (MPP) is a model cation that is transported by OCT1, OCT2 and OCT3 from various species and exhibits high maximal uptake rates and similar Michaelis Menten K_m values (Table IV). A variety of cations (for example tetrapentylammonium, decynium 22 and disprocynium), noncharged compounds (for example corticosterone, deoxycorticosterone, and β-estradiol) and anions (for example probenecid and α-ketogluatarate) inhibit OCTs but are not transported themselves (Table II, III and IV). Although the substrate and inhibitor specificities of OCT1, OCT2 and OCT3 broadly overlap there exist differences in specificity between individual subtypes and between identical subtypes of different species (6). Some cations are transported by one OCT-transporter and are non-transported inhibitors of another OCT-transporter. For example tetrabutylammonium is transported by hOCT1 and OCT1 from rabbit (rbOCT1) but is a non-transported inhibitor of rOCT1, mOCT1 and rOCT2 (93,97,98). The affinities of transported substrates and non-transported inhibitors for individual OCT transporters overlap broadly (Tables II, III and IV). This shows that selectivity of cation binding versus cation transport is determined by different criteria (88). Transported substrates of the OCTs exhibit mutual inhibition. The degree of inhibition by a high concentration of a given inhibitory substrate may be total or partial. The inhibition may be competitive, however, deviations from classical competitive type of inhibition were also observed for some pairs of substrate and inhibitor (Koepsell H., unpublished data).

Substrate and Inhibitor Specificities of OCTs

hOCT1. Compounds that are transported by human hOCT1 include the model cations MPP, tetraethylammonium (TEA), tetrapropylammonium (TPrA), tetrabutylammonium (TBuA), N-methylquinine and *N*-(4.4-azo-*n*-pentyl)-21-deoxyajmalinium, the endogenous compounds choline, acetylcholine and agmatine, and the drugs quinidine, quinine, aciclovir, ganciclovir and metformin (14,15,47,103,107,111,116). Transported substrates of hOCT1 with the lowest K_m values are MPP (15–32 μM) and TBuA (30 μM) (103). For several drugs which inhibit OCTs but are not transported or have not been tested for transport, a higher affinity to hOCT1 was observed compared to hOCT2 or hOCT3 (Table III). These are the

Table III. Drugs that Interact with Polyspecific Organic Cation Transporters

Class	Compound	Km or (IC50) [μM][a, b]								References
		hOCT1	hOCT2	hOCT3	hOCTN1	hOCTN2	hOCT6	hMATE1	hMATE2-K	
Receptor antagonists										
Acetylcholine receptor (muscarinic)	Atropine	(1.2)	(29)	(**466**)						(50)
Acetylcholine receptor (muscarinic)	Mepiperphenidol		**4.8**							(15)
α-Adrenoceptor	Phenoxybenzamine	(2.7)	(4.9)	(6.1)						(52)
α-Adrenoceptor	Prazosin	(1.8)	(>100)	(13)						(52)
β-Adrenoceptor	Acebutolol	(96)								(107)
Histamine H_1 receptor	Diphenylhydramine	(3.4)	(15)	(695)						(50)
Histamine H_1 receptor	Pyrilamine				**(<100)**	**(<500)**				(99,102)
Histamine H_2 receptor	Cimetidine	**(166)**	**8.6, 73 (70)**	(17)	(435)	(<2000)		(<20)	**370**	(36,42,99,100,102, 103,107,109)
Histamine H_2 receptor	Famotidine		**204 (111)**							(50,103)
Histamine H_2 receptor	Ranitidine	(28)	**265 (40)**	(372)						(50,103,109)
NMDA receptor	Amantadine	(236)	**20, 27 (28)**	(>1000)						(63,103,105)
NMDA receptor	Ketamine	(115)	(23)	(226)						(105)
NMDA receptor	Memantine	(3.7)	**34 (7.3)**	236						(63,105)
NMDA receptor	Phencyclidine	(4.4)	(25)	(333)						(105)
Receptor agonists										
Acetylcholine receptor (nicotinic)	Nicotine	(186)	(42)	(101)	(>5,000)	(>1,000)		(>500)		(7,47,99,102)
α-Adrenoceptor	Clonidine	(0.6 – 6.5)	(23)	(110, 373)	(<5,000)	(<3,000)				(23,50,99,102, 103,107)
α-Adrenoceptor	Etilefrine	(447)	(4,009)	**2800 (4450)**						(50)
β-Adrenoceptor	O-Methylisoprenaline	(>100)	(570)	(4.4)						(15,52)
NMDA receptor	Dizocilpine	(81)	(22)	(224)						(105)
Ion channel and transporter blockers										
Ca^{2+}channel	Verapamil	(2.9)	(206)[c]	(24)	(**8.4**)	(**<50**)		(<50)	substrate	(7,36,42,99,102,107)
Na^{+}channel	r (−) Disopyramide	(15)								(107)
Na^{+}channel	s (+) Disopyramide	(30)								(107)
Na^{+}channel	Procainamide	(74)	(50, 58)	(738)	(<500)	(<3000)	(<12,000)	(<500)	**4,100**	(7,23,42,87,99, 102,107)
Na^{+}channel	Quinidine	**(18)**			(**69**)	(**<100**)		(<10)	substrate	(7,36,42,99,101, 102,107)
Noradrenaline transporter	Cocaine	(85)	(113, 277)[c]	(>1000)						(105)
Serotonin transporter	Citalopram	(2.8)[c]	(21)[c]	(158)[c]						

Drugs and Xenobiotics									
Anesthetic	Midazolam	(3.7)							(107)
Anesthetic	Lidocaine				(0.8)		(<12,000)		(87)
Antiasthmatic	Beclometasone		(4.4)						(47)
Antiasthmatic	Budesonide		(7.3)						(47)
Antibiotic	Cefepine					(1,700)			(110)
Antibiotic	Cefoselis					(6,400)			(110)
Antibiotic	Cefsulodine					(<500)			(99)
Antibiotic	Cephaloridine				(<1,000)	**230**			(102,110)
Antibiotic	Levofloxacine				(>5,000)				(102)
Antibiotic	Ofloxacine				(>5,000)				(102)
Antidepressant	Desipramine	(5.4)	(16)	(14)					(15,23,107,111)
Antidepressant	Imipramine			(42)					(23)
Antidiabetic	Metformin	**1,470 (2,010)**	**(339, 1,700)990**					**1,050**	(42,103,112,118)
Antidiabetic	Phenformin	(10, 153)	(65)						(103,118)
Antiemetic	Emetine					**4.2 (<300)**			(99,101)
Antiemetic	Granisetron	(<100)[c]	(<100)[c]	(<100)[c]					
Antiemetic	Odansetron	(<100)[c]	(<100)[c]						
Antiemetic	Tropisetron	(<100)[c]	(<100)[c]	(<100)[c]					
Antihypertensive	Debrisoquine		**(7.3)**[c]						
Antimalarial	Quinine	**(13, 23)**	**(23, 34)**	(37)	(<500)	(<100)			(42,71,99,102,107)
Antioxidant	Ergothioneine				**21**				(108)
Antiviral	Aciclovir	**151**							(111)
Antiviral	Ganciclovir	**516**							(111)
Antiviral (HIV)	Aquinavir	(8.3)							(113)
Antiviral (HIV)	Indinavir	(62)							(113)
Antiviral (HIV)	Nelfinavir	(22)							(113)
Antiviral (HIV)	Ritonavir	(5.2)							(113)
Cytostatic	Actinomycin D					(<500)			(99)
Cytostatic	Cisplatin	(>100)	**(1.5)**						(114)
Cytostatic	Doxorubicin						5.2		(87)
Cytostatic	Daunorubicin						<50		(87)
Cytostatic	Mitoxantrone	(16)[c]	(800)[c]	(440)[c]					
Muscle relaxant	Vecuronium	(127, 232)							(14,107)
Psychostimulant	3,4Methylenedioxymetamphetamine	(24)	(1.6)	(74)					(105)
Psychostimulant	D-Amphetamine	(202)	(11)	(460)					(105)
Serine protease inhibitor	Nafamostatmesilate		**(20)**						(115)

[a] K_m and IC_{50} values were measured in oocytes of *Xenopus laevis* or epithelial cell lines after expression of the transporters.
[b] Bold face indicates cations for which transport has been demonstrated.
[c] Koepsell et al., unpublished data.

Table IV. Model Cations and Inhibitors of Polyspecific Organic Cation Transporters

Compound	K_m or (IC_{50}) [μM][a, b]								References
	hOCT1	hOCT2	hOCT3	hOCTN1	hOCTN2	hOCT6	hMATE1	hMATE2-K	
MPP	**15, 32**	**19, 78 (24)**	**47 (54)**		(>1,000)	(<12,000)	(>100)	**94**	(14,15,23,42,87, 99,103,116)
TEA	**229 (158, 173)**	**76 (48–270)**	**(1,372)**[c]	**195–1,280**	**300**	64	**220**	**830**	(7,14,15,23, 31,36,42,79, 87,103,107, 117,118)
N^1-methylnicotinamide	(7,700)	**340 (270)**	(3,000)[c]	(77)		(<12,000)	>500		(7,15,36,42,87, 103,107)
Tetramethylammonium	(12,400)	(180,525)							(15,93,103,118)
Tetrapropylammonium	**(102)**	(20, 128)							(93,103,118)
Tetrabutylammonium	**(30)**	(20, 120)		(535)					(36,93,103,118)
Tetrapentylammonium	(1.5, 7.5)	(1.5, 11)	4.5[c]	(70)					(15,36,103,107)
Decynium 22	(2.7–4.7)	(0.10–1.1)	(0.09,0.1)						(14,15,52,107)
Disprocynium 24			(0.015)						(21)

[a] K_m and IC_{50} values were measured in oocytes of *Xenopus laevis* or epithelial cell lines after expression of the transporters.
[b] Bold face indicates cations for which transport has been demonstrated.
[c] Koepsell et al., unpublished data.

glutamate receptor antagonist phencyclidine (105), the antagonists of histamine receptors diphenylhydramine and ranitidine (50,103,109), the antagonist of the muscarinic acetylcholine receptor atropine (50), and the antidepressant desipramine (15,23,107,111). Some cations that are transported by hOCT2 and hOCT3 (epinephrine, norepinephrine, and histamine) are not transported by hOCT1 (Table II). The inhibitors of hOCT1 with the highest affinity are atropine (IC_{50} 1.2 μM) and prazosin (IC_{50} 1.8 μM) (Table III).

hOCT2. hOCT2 translocates several cations that are also transported by hOCT1. For example hOCT2 translocates MPP, TEA, quinine, and metformin with similar K_m values (Tables III and IV) and translocates acetylcholine with an about 4fold lower K_m value as hOCT1 (Table II). Uptake by hOCT2 could be also demonstrated for choline, the neurotransmitters dopamine, norepinephrine, epinephrine, serotonin, histamine, agmatine (Table II), for the glutamate receptor antagonists amantadine and memantine, for the histamine H_2 receptor antagonists cimetidine, famotidine and ranitidine, for the cytostatic cisplatin, and for the antihypertensive drug debrisoquine (Table III). Transport by hOCT2 has been described for much more cations compared to hOCT1 and hOCT3 because the analysis of transport activity by electrical measurements turned out to be much easier using *Xenopus laevis* oocytes expressing hOCT2 compared to hOCT1 and hOCT3 since higher cation induced currents were observed with this transporter. For cations that are not available as radioactively labelled compounds, high-pressure liquid chromatography or other analytical methods have to be employed to measure uptake into cells expressing OCTs.

hOCT3. Similar K_m values were determined for the translocation of MPP by hOCT1, hOCT2 and hOCT3 whereas a much higher K_m value was measured for translocation of TEA by hOCT3 compared to hOCT1 and hOCT2

Table V. Cations that can be used to Differentiate between OCT1, OCT2 and/or OCT3 of Rat or Mouse

Species	Compound	K_m or (IC_{50}) [μM][a, b]			References
		OCT1	OCT2	OCT3	
rat	Corticosterone	(151)	(4.0, 4.2)	(4.9)	(72,90)
	Dopamine	**19, 51**	**2,100, 2,300, 3,600**	(620)	(72,106,119)
	Estradiol	(35)	(85)	(1.1)	(72)
	Memantine	(1.7)	(73)	(295)	(119)
	O-Methylisoprenaline	(37)	(2,600)		(90)
	Norepinephrine		(4.400, 11.000)	(432)	(72,106)
	Serotonin	**38**	(3.600)	(970)	(72,106,119)
mouse	Cimetidine	(0.59)	(8.0)	(1.3)[c]	(120)
	O-Methylisoprenaline	(8.4)[c]	(>100)[c]	(1.4)[c]	
	Procainamide	(3.9)	(312)	(11)[c]	(120)
	Quinine	(0.28)	(2.8)	(3.0)[c]	(120)

[a] K_m and IC_{50} values were measured in oocytes of *Xenopus laevis* or epithelial cell lines after expression of the transporters.
[b] Bold face indicates cations for which transport has been demonstrated.
[c] Koepsell et al., unpublished data.

(Table IV). A much lower affinity for the inhibition of hOCT3 compared to hOCT1 and hOCT2 was observed for amantadine, memantine, phenylcyclidine, clonidine, diphenylhydramine, atropine, procainamide and cocaine (Table III). Among the substrates of hOCT3 the highest affinities were obtained for MPP (47 μM) and histamine (180–220 μM) (Tables II, IV). High affinity inhibitors of hOCT3 are disprocynium 24 (IC_{50} value 0.015 μM), decynium 22 (IC_{50} value 0.1 μM), and corticosterone (IC_{50} value 0.12–0.29 μM) (Tables II, IV).

Compounds with Different Affinities to OCT Subtypes

To differentiate between transport activities by OCT1, OCT2 and OCT3 in human cell lines or in rats and mice, inhibitors with largely different affinities for OCT1, OCT2 and/or OCT3 can be employed. To differentiate between transport by human OCTs we suggest to measure MPP uptake and to use amantadine (IC_{50} values: hOCT1 236 μM, hOCT2 20–28 μM, hOCT3 > 1,000 μM), phencyclidine (IC_{50} values: hOCT1 4.4 μM, hOCT2 25 μM, hOCT3 330 μM), diphenylhydramine (IC_{50} values: hOCT1 3.4 μM, hOCT2 15 μM, hOCT3 695 μM) and/or atropine (IC_{50} values: hOCT1 1.2 μM, hOCT2 29 μM, hOCT3 466 μM) as inhibitors. Since the affinity for the interaction of individual compounds with OCTs may be dependent on experimental conditions employed for the uptake measurements (Koepsell, H., Gorboulev, V., Volk, C., unpublished data), we recommend to use at least two different inhibitors.

In Table V compounds are listed that inhibit uptake of MPP by OCT1, OCT2 and/or OCT3 from rat or mouse with different affinities. Memantine may be used to differentiate between uptake by rOCT1, rOCT2 and rOCT3. Dopamine and corticosterone may be used to distinguish cation uptake by rOCT1 and by rOCT2 or rOCT3. Estradiol is helpful to distinguish between uptake by rOCT2 and rOCT3, and O-methylisoprenaline can be used to distinguish between uptake by rOCT1 and rOCT2. In mice O-methylisoprenaline may be used to differentiate between cation uptake by mOCT1, mOCT2 and mOCT3, procainamide or cimetidine may be used to differentiate between uptake by mOCT1 or mOCT3 from uptake by mOCT2 whereas quinine may be used to differentiate between uptake by mOCT1 from uptake by mOCT2 or mOCT3.

Function and Substrate Specificities of Members of SLC22 Family that Translocate Carnitine as well as Organic Cations

hOCTN1. Human OCTN1 (hOCTN1) transports the zwitterionic antioxidant ergothioneine with relatively high affinity ($K_m = 21$ μM) (108). In addition, it transports the zwitterions L-carnitine and stachydrine and the organic cations TEA, quinidine, pyrilamine and verapamil (Tables II, III, and IV, (31,36,102)). It has been shown that hOCTN1 is inhibited by many additional compounds. For example TEA uptake by OCTN1 was inhibited by choline, L-carnitine, D-carnitine, cephaloridine, cimetidine, clonidine, levofloxacine, lidocaine, ofloxacine, procainamide, quinine, quinidine, tetrabutylammonium, tetrapentylammonium, nicotine, and verapamil (31,79,102). High affinity interaction was obtained for L-carnitine ($K_i = 24$ μM), verapamil ($K_i = 8.4$ μM) and lidocaine (0.83 μM) (36). Apparently, hOCTN1 employs different translocation mechanisms for different substrates and is able to operate in both directions. When hOCTN1-expressed TEA uptake was measured in membrane vesicles in the presence of an initial outwardly directed proton gradient, a transient increase of the intravesicular TEA concentration over the equilibrium value was observed (79). Such an "overshoot" indicates energetic coupling between efflux of protons and influx of TEA indicating an antiport transport mechanism. At variance, hOCTN1-mediated uptake of the zwitterion ergothioneine was stimulated by extracellular sodium whereas uptake of the zwitterion stachydrine was inhibited by extracellular sodium (108). Since murine OCTN1 was localized to the luminal brush-border membrane of renal proximal tubules and hOCTN1 is expressed in epithelial cells of renal proximal tubules and small intestine, OCTN1 is supposed to be involved in the secretion of cations in renal proximal tubules and small intestine. In addition, OCTN1 is supposed to mediate the absorption of ergothioneine in small intestine and the reabsorption of ergothioneine in the kidney. The antioxidant ergothioneine is present in plants and mushrooms and reaches high concentrations in bone marrow and erythrocytes. It has been speculated that ergothioneine may protect erythrocytes and monocytes against oxidative damage (108).

hOCTN2. Functional characterization of transport in cells overexpressing hOCTN2 revealed that hOCTN2 is a Na^+-dependent, high affinity transporter for L-carnitine, acetyl-L-carnitine and the zwitterionic β-lactam antibiotic cephaloridine, but can function alternatively as a polyspecific and Na^+-independent cation transporter (80,83,99,100,101,110,121). Cationic substrates of hOCTN2 are TEA, choline, verapamil and pyrilamine. Inhibition of hOCTN2 mediated uptake has been demonstrated for cimetidine, clonidine, procainamide, actinomycin D, quinine, emetine, cefsulodine, nicotine, MPP (Tables II, III and IV). Na^+-dependent transport of L-carnitine by OCTN2 is electrogenic and stereospecific (99,101) and Na^+ increases the affinity for L-carnitine (122). Uptake measurements with plasma membrane vesicles of HEK-293 cells in which hOCTN2 was overexpressed, provided evidence that hOCTN2 is a Na^+-L-carnitine cotransporter, showing that an initial inwardly directed Na^+ gradient induced a transient increase of intravesicular L-carnitine concentration beyond the final equilibrium value (83). Together with the demonstration of electrogenicity, this "overshoot" phenomenon provided evidence that hOCTN2 is a Na^+-L-carnitine cotransporter rather than a transporter that is activated by extracellular Na^+. For human OCTN2, an apparent K_m for L-carnitine of 4–5 μM (80,101), and half-maximal concentrations for Na^+-activation between 2 and 19 mM (99,101,123) were determined. Since the functional characteristics of L-carnitine uptake by OCTN2 are very similar to those determined for L-carnitine transport into plasma membrane vesicles isolated from skeletal muscle, kidney and intestine (124–126) the data suggest that OCTN2 is responsible for L-carnitine uptake into small intestinal epithelial cells, renal epithelial cells and skeletal muscle cells.

It has been shown that TEA and L-carnitine interact at the same binding site of OCTN2 and/or use the same transport path since carnitine uptake by hOCTN2 was competitively inhibited by TEA, and TEA uptake by hOCTN2 was competitively inhibited by L-carnitine (117,123). A detailed characterization of the mouse OCTN2 (mOCTN2)

showed that mOCTN2 translocates organic cations in either direction across the plasma membrane (121). L-carnitine uptake was *trans*-stimulated by intracellular TEA, and TEA efflux was *trans*-stimulated by extracellular L-carnitine (121).

OCTN3. This subtype has been only cloned from mice (mOCTN3). At variance to OCTN2 transporters from different species mOCTN3 transports carnitine independently of Na^+. For L-carnitine transport by mOCTN3 an apparent K_m of 3 μM was determined. This value is 7-fold lower compared to the apparent K_m value of L-carnitine transport by mOCTN2 (32). Given that carnitine uptake by mOCTN3 was not inhibited by 0.5 mM choline, and inhibited only by 54% by 0.5 mM TEA, mOCTN3 appears less relevant quantitatively for organic cation transport than mOCTN1 and mOCTN2. Since mOCTN3 was localized to the basolateral membrane of small intestinal enterocytes (85) we speculate that mOCTN3 is localized in the basolateral membrane of renal proximal tubules and mediates basolateral efflux of L-carnitine.

OCT6 or CT2 (SLC22A16). OCT6 is a high affinity carnitine transporter that also translocates various organic cations such as TEA and doxorubicin (37,87). Whereas uptake of L-carnitine was partially dependent on the presence of sodium, uptake of doxorubicin was sodium independent. OCT6 appears to be polyspecific since TEA uptake was inhibited by various other organic cations (Tables II, III and IV).

Function and Specificities of MATE Transporters

MATE1. Human MATE1 (hMATE1) mediates uptake of TEA with a K_m value of 220 μM. TEA uptake by hMATE1 was independent of the membrane potential and the extracellular concentration of sodium. Since hMATE1-mediated uptake and efflux of TEA was stimulated by in>out or out>in proton gradients, respectively, MATE1 is supposed to be a cation-proton antiporter that operates in both directions (7). TEA uptake by MATE1 was inhibited by a large variety of organic cations such as MPP, serotonin, cimetidine, quinidine, and verapamil suggesting a polyspecificity similar to the OCT- and OCTN-transporters (Tables II, III, IV). Interestingly, corticosterone, progesterone and testosterone inhibited hMATE1-mediated uptake of TEA with relatively high affinity (Table II). A characterization of cation transport by MATE1 from rat (rMATE1) revealed that rMATE1 is a proton-cation antiporter that translocates TEA with a similar affinity as hMATE1 (41). In addition, it was shown that rMATE1 translocates cimetidine with a K_m value of 3 μM.

MATE2. So far human MATE2-K (hMATE2-K) is the only MATE2 protein for which transport activity has been demonstrated (42). hMATE2-K is located in the brush-border membrane of renal proximal tubules. It is a polyspecific proton–cation antiporter that translocates TEA, cimetidine, MPP, procainamide, metformin and N^1-methylnicotinamide, creatinine, guanidine, quinidine, thiamine, and verapamil.

Regulation

To understand the physiological and biomedical significance of polyspecific organic cation transporters their regulation during short term and long term adaptations must be understood. This will help to develop drugs that modify the excretion of xenobiotics. It turned out that the regulation of transporters is complex. It may occur at the transcription, message stability, translation and various posttranscriptional levels. Regulation may be different for subtypes and orthologues of transporters and may vary for an individual transporter in different tissues.

Short Term Regulation of OCT1-3

Short term regulation of OCT1, OCT2 and OCT3 was studied in HEK293 cells and CHO cells that were stably transfected with hOCT1, hOCT2, hOCT3 and rOCT1 (127–131). With hOCT1 expressed in HEK293 cells or CHO cells similar results were obtained, however, distinct differences in regulation were observed for the three human subtypes. In addition, distinct differences were observed for the regulation of OCT1 from human and rat. PKA inhibits uptake of the fluorescent organic cation 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium iodide (ASP) expressed by hOCT1 and hOCT2 but does not alter MPP uptake expressed by hOCT3 (127,130,131). At variance, cation uptake expressed by hOCT1, hOCT2 and hOCT3 is stimulated by the Ca^{2+}/calmodulin (CaM) pathway (130,131). Surprisingly, certain characteristics observed for regulation of ASP transport across the basolateral membrane of human renal proximal tubules were not found measuring ASP uptake by hOCT2 or hOCT3 expressed in HEK293 cells or CHO cells. Note that hOCT2 and hOCT3 are located in the basolateral membrane of renal proximal tubules. For example, PKC that inhibits basolateral ASP uptake in human proximal tubules (132), has no effect on ASP uptake expressed in HEK293 cells by hOCT2 and of MPP uptake by hOCT3 (127,130,131). Importantly, regulation of OCTs may affect their affinity for certain substrates and thereby change the substrate selectivity. For example, inhibition of the Ca^{2+}/CaM complex by calmidazolium decreased the affinity for TEA of hOCT1 and hOCT2 expressed in HEK293 cells sixfold and tenfold, respectively (127,130). The interaction of hOCT1, hOCT2 and hOCT3 with PDZ (*P*SD95, *D*lg, *Z*O1) domains and with proteins containing PDZ domains such as the proteins NHERF1, NHERF2, PDZK1, IKEPP was investigated (133,134). It was observed that the C-terminus of hOCT3 interacted with the PDZ domain protein IKEPP that is expressed in intestine and kidney.

Short term regulation of rOCT1 has been investigated in detail (128,129,135). In HEK293 cells stably transfected with rOCT1, transport of ASP was stimulated by activators of PKC, PKA, and tyrosine kinase (128). rOCT1 was phosphorylated after PKC-dependent stimulation (128). After stimulation of rOCT1 by PKC, the IC_{50} values obtained for inhibition of ASP uptake by TEA or tetrapentylammonium (TPeA) were decreased by factors of 58 and 15, respectively (128). PKC dependent stimulation of ASP uptake by rOCT1 was abolished when individual serine or threonine residues in each of the five putative PKC phosphorylation sites were replaced by alanine (129). After mutations of the PKC phosphorylation sites, no effect of PKC on the affinity of TEA and TPeA on the inhibition of ASP uptake could be detected.

Long Term Regulations of OCT1-3 under Physiological Conditions

At variance to OCT1 and OCT3, the expression of OCT2 is gender dependent and regulated by steroid hormones. TEA uptake in renal cortical slices of male rats was greater compared to female rats and the amounts of rOCT2 mRNA and rOCT2 protein in male rat kidneys were greater compared to females (136,137). The higher expression of rOCT2 in male rats compared to female rats correlated with basolateral uptake of TEA into rat renal proximal tubule cells. Application of testosterone to female rats resulted in increased renal expression of OCT2, whereas application of estradiol to male rats decreased the renal expression of OCT2 (138). Similarly mRNA of OCT2 in Mardin Darby canine kidney (MDCK) cells was increased about 2fold following exposure to testosterone (139). Dexamethasone and hydrocortisone had a similar effect. Recently it was shown that the androgen receptor is involved in the transcriptional upregulation of rOCT1 by testosterone (140). In the promotor of rOCT2 two androgen response elements were identified that are required for the testosterone-dependent regulation of rOCT2. The steroid hormone dependent regulation of OCT2 is species dependent. In kidneys of male rabbits the concentration of rbOCT2 mRNA was higher compared to female kidneys, similar to rats, however, no significant difference between males and females could be detected in renal rbOCT2 protein and in TEA uptake across the basolateral membrane of renal proximal tubules (141).

The promotor of hOCT1 contains two DNA response elements for the hepatocyte nuclear factor-4α (HNF-4α) (142). HNF-4α interacts with these response elements and activates transcription of hOCT1. Activation of hOCT1 by HNF-4α was inhibited by the bile acid chenodeoxycholic acid via a component (SHP, small heteromeric partner) of the bile acid-inducible transcriptional repressor (142). In the promotor of mouse OCT1 a peroxisome proliferator agonist receptor (PPAR) response element was identified and transcriptional upregulation of OCT1 by agonists of PPAR-α and -γ such as clofibrate and ciglitazone was demonstrated (143). PPAR agonists are commonly employed for the control of dyslipidemias and diabetes mellitus.

Long Term Regulations of OCT1-3 under Pathologic Conditions

The expression of OCTs may be changed during diseases and after treatment with drugs. (1) It was observed that OCT2 in rat the kidney was decreased during chronic renal failure. In 5/6-nephrectomized rats, a model for chronic renal failure, renal clearance of cimetidine was decreased in parallel with the expression of rOCT2 protein in the nephrectomized kidneys whereas the expression of rOCT1, rOAT1, rOAT3 and the $(Na^+ + K^+)$-ATPase was not changed (144). Since the plasma concentration of testosterone was decreased after partial nephrectomy and the expression of rOCT2 in nephrectomized kidneys was increased after administration of testosterone, decrease of plasma testosterone during chronic renal failure may be an etiologic factor for the downregulation of OCT2. (2) Renal expression of OCTs may be decreased during diabetes. It was shown that streptozotocin-induced diabetes in rats was associated with a reduction of renal clearance of N^1-methyl-nicotinamide (NMN), basolateral uptake of TEA into renal proximal tubules and renal protein expression of rOCT1, rOCT2 and rOCT3 (145–147). (3) Renal expression of OCT2 is decreased during hyperuricemia. When rats were fed 10 days with oxonic acid, an inhibitor of uric acid metabolism, plasma uric acid level, plasma creatinine and blood urea nitrogen were significantly increased. In parallel, TEA uptake in renal slices, rOCT2 mRNA and rOCT2 protein in the kidney were decreased (148). (4) Hepatic expression of OCT1 is decreased during cholestasis. After bile duct ligation in rat, OCT1 protein decreased in liver but not in kidney (149). In parallel, hepatic accumulation of intravenously injected TEA was decreased. We speculate that increased levels of bile acids after obstructive cholestasis in humans may inhibit HNF-4α-mediated activation of OCT1 transcription. Cholestasis can be also induced by intraperitoneal application of lipopolysaccharides mimicking effects of cell wall components of gram-negative bacteria. After treatment of rats with lipopolysaccharides many drug transporters expressed in the liver including OCT1 were downregulated (150). (5) During treatment of rats with methamphetamine the expression of OCT3 in brain is decreased suggesting a drug-induced downregulation (151). At variance, data were obtained suggesting that OCT1 and OCT3 are upregulated in transgenic mice that did not contain the Na^+-serotonin cotransporter SERT (43,44).

Regulation of OCTN1–3

Transcription of the *SLC22A4* gene expressing hOCTN1 is inhibited by the transcription factor RUNX1 that binds to a consensus sequence in the the first intron of the *SLC22A4* gene (75). RUNX1 is mainly expressed in hematopoetic cells. It functions both to activate and repress transcription through interaction with cofactors. By stimulation of cultivated synoviocytes expressing hOCTN1 with TNFα, levels of *SLC22A4* mRNA were increased twofold (75). A promotor analysis of the murine gene encoding mOCTN3 revealed that binding sites of the transcription factor Sp1 are necessary for constitutive expression (152). The expression of mOCTN3 was enhanced by palmic acid and the transcription factor AP-1 appeared to be involved in this regulation.

Targeting of OCTN1 and OCTN2 to the brush border membrane of enterocytes and/or regulation of these transporters is influenced by proteins with PDZ domains (133,134). hOCTN1 and hOCTN2 interact with PDZ domains. It has been shown that the four N-terminal amino acids of OCTN2 bind to the PDZ domain of PDZK1 that is located at the brush-border membrane (134). Since L-carnitine uptake was increased when PDZK1 was coexpressed with OCTN2 but the concentration of OCTN2 in the plasma membrane was not changed, PDZK1 may be involved in the regulation of OCTN2 activity.

Investigating the regulation of hepatic transporters after 2/3 hepatectomy in rats it was observed that OCTN1 and

OCTN2 belonged to a large group of transporters that were upregulated during liver regeneration (153).

MICE WITH TARGETED DISRUPTION OR DEFECT MUTANTS

Targeted Disruption of OCTs

Knockout mice for OCT1, OCT2, and OCT3, and a double knockout mouse for OCT1 and OCT2 have been generated (154–156). The strains were fertile and showed no obvious physiological defect. After intravenous injection of TEA, MPP, metformin, cimetidine and/or choline, the biliary excretions and renal excretions and tissue distributions of the respective compounds were determined (154–157). In *Oct1*-knockout mice, the concentrations of TEA, MPP and metformin in liver and small intestine were reduced (154,157). The biliary excretion of TEA which is a good substrate of mOCT1 and mOCT2 but is poorly transported by mOCT3 was decreased whereas the renal TEA excretion was increased. This indicates the critical role of OCT1 for TEA excretion in the liver and suggests a compensatory upregulation of renal TEA excretion. In *Oct2*-knockout mice the concentration of TEA in liver and kidney and the excretion of TEA in the urine and feces were not changed (156). At variance, in *Oct1* and *Oct2* double-knockout mice the renal excretion of TEA was drastically reduced. The data indicate that renal excretion of TEA in mice is mainly mediated by OCT1 and OCT2. They suggest that OCT3 which is also expressed in liver and kidney does not contribute significantly to the excretion of TEA. Pharmacokinetic studies in *Oct3*-knockout mice using radioactively labelled MPP did not reveal significant changes of MPP concentrations in small intestine, liver, kidney, brain, and placenta (155). In contrast, the concentration of MPP was significantly decreased in heart. Since mOCT1 and mOCT2 are also expressed in heart, and MPP is well transported by mOCT1, mOCT2 and mOCT3, the data indicate that mOCT3 is the most important OCT-transporter in heart. After intravenous application of MPP to pregnant *Oct3*-knockout mice the MPP concentration in the embryos was reduced whereas the MPP concentration in the placenta remained unchanged. These data suggest that OCT3 is not critical for passage of organic cations across the materno-fetal barrier but important for the uptake of organic cations into embryonic tissues. *Oct3*-knockout mice showed an increased ingestion of hypertonic saline under thirst and salt appetite conditions suggesting the importance of OCT3 for salt-intake regulation (74).

Defect Mutant of OCTN2

Juvenile visceral steatosis (*jvs*)-mice contain a homozygous loss-of-function mutation of OCTN2 that causes "primary systemic carnitine deficiency" (SCD) (158–160). Because intracellular carnitine is required for transferring long-chain fatty acids from the cytosol into mitochondria, the oxidation of fatty acids is disturbed in SCD. *Jvs* mice suffer from symptoms such as lipid accumulation in the liver, cardiac hypertrophy, hypoglycemia, and hyperammonemia (161–164). *Jvs* mice may suffer from obstruction of the distal part of the epididymis (165).

Mutations of Polyspecific Organic Cation Transporters in Man and Genetic Diseases

To determine whether difference in absorption, excretion or tissue distribution of cationic drugs may be caused by polymorphisms in the *SLC22A1-3* genes encoding hOCT1-3 different ethnic populations were screened for polymorphisms (166–173). Mutations in the *SLC22A5* gene encoding OCTN2 have been identified to cause systemic carnitine deficiency (174–176). Data have been presented showing a correlation between mutations in the *SLC22A4* and *SLC22A5* genes encoding hOCTN1 and hOCT2 and autoimmune diseases (36,177–180).

Polymorphisms in the *SLC22A1* Gene Encoding hOCT1

After sequencing the *SLC22A1* gene from 667 individuals of various ethnic backgrounds many single-nucleotide polymorphisms (SNPs) with population dependent frequencies were observed (166–168,172,173). 18 SNPs result in single amino acid substitutions or the deletion of one amino acid. For six mutations (Arg61Cys, Cys88Arg, Gly220Val, Pro341Leu, Gly401Ser, Gly465Arg) reduced transport activities were observed, and for one mutation (Ser14Phe) expressed transport was increased. For mutations Cys88Arg, Gly220Val, Gly401Ser, and Gly465Arg respective frequencies of 0.01%, 0.2%, 0.8%, 1.6%, were obtained and transport of MPP was reduced to <10% compared to wildtype (166,167). For mutants Cys88Arg and Gly401Ser the substrate selectivity was changed (166). So far biomedical consequences of the identified defect mutations in human OCT1 have not been identified.

Polymorphisms in the *SLC22A2* Gene Encoding hOCT2

Sequencing the *SLC22A2* gene in 366 individuals of different ethnicity ten variants were identified that caused amino acid changes (169–171). One variant was detected that leads to a premature stop codon at amino acid position 48. Four of the nonsynonymous variants (Ala270Ser, Met165Ile, Arg400Cys and Lys432Gln) showed ethnic-specific gene frequencies >1%. All variants were functionally active and showed no or no dramatic functional difference compared to wildtype hOCT2. Mutants Met165Ile and Arg400Cys may have a higher V_{max} and mutants Met147Val, Ala270Ser and Lys432Gln may have a slightly differing substrate selectivity (170,171). Interestingly, a recent study suggests that individuals with the Ala270Ser allele less frequently suffer from hypertension compared to homozygous carriers of the wildtype allele Ala270 (181).

Polymorphisms in the *SLC22A3* Gene Encoding hOCT3

In the *SLC22A3* gene so far no mutations have been reported that lead to an exchange of amino acids. A comparison of single nucleotide polymorphisms (SNPs) in the *SLC22A3* gene in 213 persons with methamphetamine use disorder and 443 healthy controls suggested a correlation between SNPs in the *SLC22A3* gene and methamphetamine

dependence (182). These SNPs may be involved in the regulation of expression.

Mutations and Polymorphisms in the *SLC22A4* Gene Encoding hOCTN1

Mutations in the *SLC22A4* and *SLC22A5* genes localized in the 5q31 cytokine gene cluster were associated with autoimmune diseases such as Crohn disease, rheumatoid arthritis, and/or ulcerative colitis (36,75,177,178,180). A mutation in the *SLC22A4* gene resulting in the exchange of leucine in position 403 by phenylalanine was associated with Crohn disease (36). This mutation leads to an alteration of substrate selectivity of hOCTN1 and to a decrease of maximal velocity of TEA uptake (36,183). It may be speculated that changes in intracellular concentrations of OCTN1 substrates such as carnitine and ergothioneine may influence the lipid metabolism and thereby change susceptibility to autoimmune diseases (183–185). Interestingly, the Leu403Phe mutant of hOCTN 1 shares an epitope with *Campylobacter jejuni* and *Mycobacterium paratuberculosis* that have been implicated in the pathogenesis of Crohn disease (78). Crossreactive antibodies generated after infection with these bacteria may further impair the function of Leu403Phe mutant of hOCT1 in intestine. The Leu403Phe mutant of hOCTN1 may be also correlated with an increased risk for diabetes mellitus type I (186).

Mutations and Polymorphisms in the *SLC22A5* Gene Encoding hOCTN2

Genetic variations in the *SLC22A5* gene that cause low expression or strongly impaired function of hOCTN2 lead to systemic carnitine deficiency (SCD), a recessively inherited disorder of mitochondrial fatty acid oxidation (174–176). Because intracellular carnitine is required for transferring long-chain fatty acids from the cytosol into the mitochondria, the lack of carnitine caused by impaired carnitine uptake interferes with the ability to fuel metabolism via the oxidation of fatty acids. Early in life, SCD may cause acute hypoketotic hypoglycemia, Reye syndrome (encephalopathy with hyperammonemia) and sudden infant death. Later manifestations of SCD include cardiomyopathy and progressive skeletal weakness (187). 276 individuals from different ethnic groups that did not suffer from SCD were screened for mutations in the *SLC22A5* gene coding for hOCTN2. Eight amino acid exchanges were observed and three of these showed changes in activitiy or distribution (188). In the promotor of OCTN2 a SNP (−207G→C) was identified that was associated with Crohn disease and ulcerative colitis (35,177). This SNP disrupts the activiation of the hOCTN2 promotor by heat schock transcription factor I.

STRUCTURE FUNCTION RELATIONSHIPS

Substrate Binding Domains of OCTs

Using site directed mutagenesis with rOCT1 seven amino acids could be identified that are involved in cation binding (Fig. 1); tryptophan 218, tyrosine 222 and threonine 226 on successive turns of the α-helix that forms the predicted fourth transmembrane domain (TMD) (189), alanine 443, leucine 447 and glutamine 448 in the tenth TMD (190), and aspartate 475 in the middle of the 11th TMD (189,191) (Fig. 1). When Asp475 was replaced by glutamate, the maximal transport rate observed after expression in oocytes of *Xenopus laevis* was reduced to 2–4% for TEA, NMN and choline and to 11% for MPP. Interestingly, the Asp475Glu mutant exhibited 8–15 times lower K_m values for TEA and choline than wildtype OCT1 whereas the K_m value for MPP was unchanged. Two tentative conclusions were drawn from these observations: firstly, Asp475 is located within or close to the substrate binding site of rOCT1, and secondly, rOCT1 contains a binding region rather than a single binding site that offers several, only partially overlapping interaction domains for different substrates. The assignment of Ala443, Leu447 and Gln448 within or close to the substrate binding region of rOCT1 is based on the following observations: first, the low affinity of corticosterone to rOCT1 could be transformed to the 30–40 times higher affinity of corticosterone to OCT2 when Ala443, Leu447 and Gln448 in rOCT1 were replaced by corresponding amino acids of rOCT2 (Ala443Ile, Leu447Phe and Gln448Glu). Secondly, replacement of Leu447 and Gln448 (Leu447Phe, Gln448Glu) resulted in a significant decrease of the K_m value for MPP whereas the replacement of Ala443, Leu447, Gln448 (Ala443Ile, Leu447Phe and Gln448Glu) lead to a decrease of the K_m value for TEA. Thirdly, Glu 447 in OCT2 from rabbit (rbOCT2) that corresponds to Glu448 in rOCT2 was shown to be partially responsible for the higher affinities of cimetidine to rbOCT2 compared to OCT1 from rabbit (192). The data indicate that corticosterone binds to the substrate binding region and supports the interpretation that the substrate binding region of rOCT1 contains partially overlapping binding sites for different substrates. The amino acids Trp218, Tyr 222 and Thr 226 on successive turns of the fourth TMD are located within the substrate binding region of rOCT1 because mutations in these positions resulted in a decrease of K_m values for individual substrates, to changes in substrate specificity and in some cases to changes in V_{max} values (189).

Recently, two members of the MFS superfamily, the lactose permease LacY and glycerol-3-phosphate transporter GlpT from *E. coli* (193,194) were crystallized and their tertiary structure determined. The structures were very similar. They show a large cleft that opens to the intracellular side and is formed by eight transmembrane α-helices. Modeling the tertiary structure of the TMDs of rOCT1 a model with a large cleft was obtained in analogy to LacY or GlpT (Fig. 1) (189). Importantly the seven amino acids that have been assigned to the substrate binding region using site directed mutagenesis, are located at a similar depth within this cleft. A comparison of the substrate binding region in the model with the sizes of substrates suggests that more than one compound can bind at the same time. Recently, the model of the tertiary structure of the organic cation transporters from the SLC22 family was further supported by the observation that cysteine 474 in the 11th TMD of hOCT2 that corresponds to cysteine 474 in rOCT1 is accessible from the aqueous phase (195).

Using the non-transported inhibitors tetrabutylammonium (TBuA) and corticosterone the substrate binding region of rOCT2 was investigated from the extracellular and intracellular side of the plasma membrane (97). TBuA and corticosterone interact with the substrate binding regions of OCTs because they compete with substrates and single point mutations within the substrate binding region that increase the affinity of transported cations also change the affinity of TBuA and corticosterone (97,189,190). TBuA and corticosterone were used to measure the short term inhibition of inward currents in intact oocytes induced by application of choline from the outside, and outward currents in giant patches induced by choline application from the inside. The experiments show that either inhibitor interacts from the intracellular side as well as from the extracellular side. Interestingly, TBuA had a significantly higher affinity from the outside compared to the inside, whereas corticosterone had a higher affinity from the inside compared to the outside.

The data suggest that the OCTs contain substrate binding regions that are accessible from extracellular and/or intracellular and exhibit similar but not identical substrate specificity from both sides. The similar substrate specificities from both sides and the large activation energy observed for transport by rOCT2 suggest that the OCTs only contain one substrate binding region that changes between an outwardly directed and an inwardly directed orientation during the transport cycle. The change in orientation of the substrate binding region during transport is supposed to be accompanied by structural changes that alter the affinity for substrates or inhibitors. Because the different ligands have different interaction sites, these affinity changes may go in different directions for different ligands. It is thus easy to imagine that competition between inhibitors and substrates within the binding region may vary considerably between individual inhibitors or substrates, and may be different from outside than from inside.

Substrate Binding Site of OCTN2

In patients suffering from SCD, homozygous mutations in various parts of hOCTN2 have been detected that caused large decreases of L-carnitine transport after heterologous expression (174,175,196–202). Whereas for most mutants the reason why they disturb expression of transport is not known, it has been shown that mutations in the TMDs 4 and 11 of OCTN2 have specific effects on the characteristics of expressed transport. This suggests that TMDs 4 and 11 contribute to the binding region and/or transport path as has been shown for rOCT1 (see above). Mutations of tyrosine 211 in the TMD 4 as well as serine 469 or proline 478 in the TMD 11 lead to inhibition of L-carnitine transport without affecting the transport of TEA (117,123) suggesting different binding domains for both substrates. Mutation of glutamate 452 to lysine in the highly conserved intracellular loop between TMD 10 and TMD 11 increased the half maximal value for Na^+ activation of carnitine transport to 187 mM as compared to 12 mM in wildtype without altering the V_{max} for carnitine transport at saturating Na^+-concentrations (202,203). This suggests that glutamate 452 is directly or indirectly involved in Na^+ binding to hOCTN2.

Functions of Polyspecific Organic Cation Transporters in Various Organs, Immune Cells, and Tumors

To understand the functional roles of individual organic cation transporters the functional interplay of transporters with overlapping specificity in individual cells, the specific function of the respective cells, and the physiologic function of the respective organ must be considered. In the following section the function of polyspecific organic cation transporters in several organs, immune cells and tumors is discussed.

Small Intestine

In small intestine cationic food components, drugs and xenobiotics are absorbed but can also be excreted (204–206). In Fig. 2 the polyspecific cation transporters in human small intestinal enterocytes are depicted. In humans, the first step in absorption of organic cations from the intestinal lumen is mediated by hOCTN2, hOCTN1 and/or hOCT3 in the brush border membrane whereas the efflux of organic cations over the basolateral membrane is probably mediated by hOCT1 (50). Cation uptake across the luminal membrane via the electrogenic transporter OCT3 is energized by the membrane potential whereas hOCTN1 and hOCTN2 mediate electroneutral cation–cation exchange. For cation efflux across the basolateral membrane via the electrogenic cation transporter hOCT1 an about tenfold ratio between the intracellular and extracellular concentration of the transported organic cation is required to overcome the membrane potential.

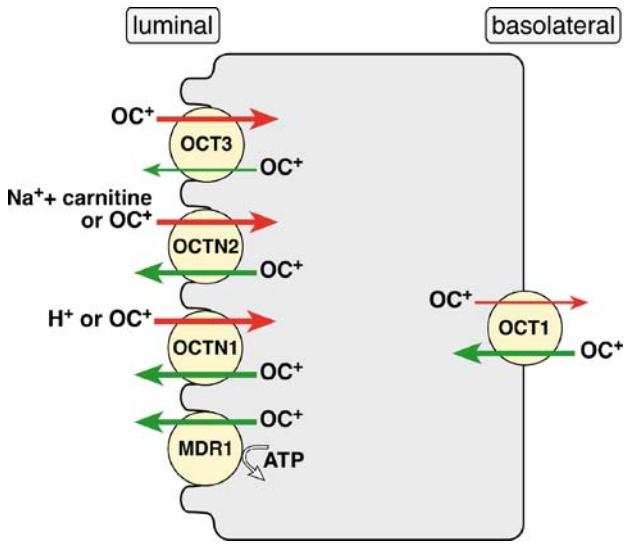

Fig. 2. Organic cation transporters in enterocytes of human small intestine. Transporter activities involved in cation absorption and cation secretion are indicated as *red and green arrows*, respectively. OCT1 and OCT3 are electrogenic cation uniporters that transport cations in both directions and are driven by the electrochemical potential. In the presence of a normal membrane potential of −60 mV cation uptake (*thick arrows*) is preferred, however, OCTs may mediate cation efflux (*thin arrows*) if the intracellular concentration of the respective cation is about ten times higher compared to the extracellular cation concentration. OCTN1 operates as secondary active proton–cation antiporter or as cation exchanger. OCTN2 can function as Na^+-carnitine cotransporter but can also mediate cation uptake or cation efflux. MDR1 is a primary active extrusion pump.

Secretion of organic cations is mediated by a combined action of hOCT1 in the basolateral membrane and several transporters in the brush border membrane. These are the organic cation/proton antiporters hOCTN1, OCTN2 that may exchange luminal carnitine plus sodium or luminal organic cations against intracellular organic cations, and the primary active efflux pump MDR1 (multidrug resistance protein) encoded by the gene *ABCB1* (207,208). MDR1 translocates hydrophobic compounds that are neutral and positively charged. Some efflux of organic cations across the luminal membrane of the enterocytes may also be mediated via hOCT3. Also in rodents, OCT1 has been localized to the basolateral membrane of the enterocytes (43). TEA uptake measurements in mice with targeted disruption of OCT1 showed that this transporter contributes significantly to the uptake of TEA across the basolateral membrane (154).

Biomedical implications. Small intestinal absorption of cationic drugs may be influenced by mutations of the involved transporters, by differences in pH of the small intestinal content, and by interaction of organic cations supplied with the food.

Liver

Most hydrophobic cations that are absorbed in small intestine enter the hepatocytes. In the hepatocytes drugs may undergo biotransformation. They are excreted across the biliary membrane into the bile or across the sinusoidal membrane back into the blood. In rat and humans, hOCT1 and hOCT3 are located in the sinusoidal membrane of the hepatocytes ((60), and unpublished data of Lips K.S., Kummer W., Ciarimboli G., Schlatter E., and Koepsell H.) (Fig. 3). These transporters mediate the first step in biliary excretion of most cationic drugs, however, they can also mediate the release of organic cations from hepatocytes into the blood. The sinusoidal membrane of hepatocytes also contains the organic anion transporting polypeptide (OATP1A2 previously called OATPA, *SLC21A3*) that translocates some organic cations and contributes to the translocation of organic cations across this membrane (4,209). After expression of OATP1A2 in *Xenopus* oocytes uptake of *N*-methylquinine and *N*-(4.4-azo-*n*-pentyl)-21-deoxy-ajmalinium has been demonstrated (209). The excretion of organic cations across the biliary membrane into the bile is mediated by the polyspecific H^+/organic cation antiporter hMATE1 and/or by P-glycoprotein MDR1 (1,7). It is possible that the H^+/organic cation transporter hOCTN1 that is also expressed in liver (Table I) also participates in biliary excretion of organic cations.

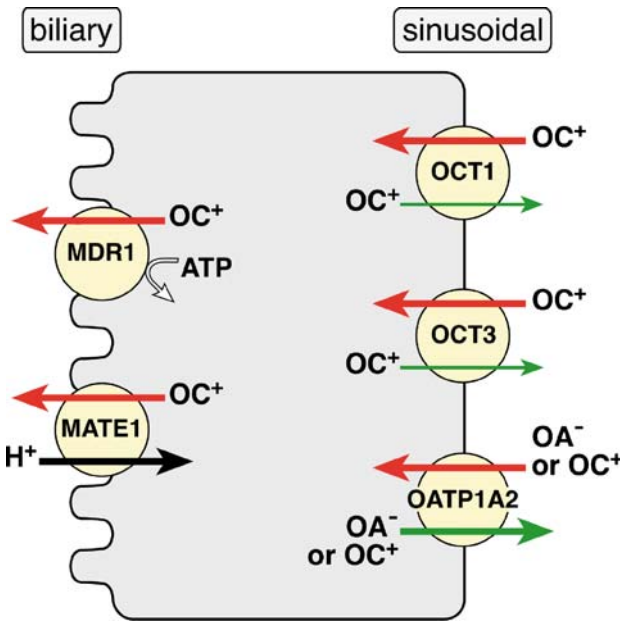

Fig. 3. Organic cation transporters in human liver. For explanation see Fig. 2. Transporter activities that mediate biliary excretion are indicated in *red*. *Green arrows* indicate the release of organic cations from hepatocytes into sinusoids. MATE1 is a secondary active proton–cation antiporter. OATP1A2 is an anion exchanger that is also able to translocate some cations. The sinusoidal localization of OCT3 was observed in unpublished experiments of K.S. Lips, W. Kummer, G. Ciarimboli, E. Schlatter., and H. Koepsell.

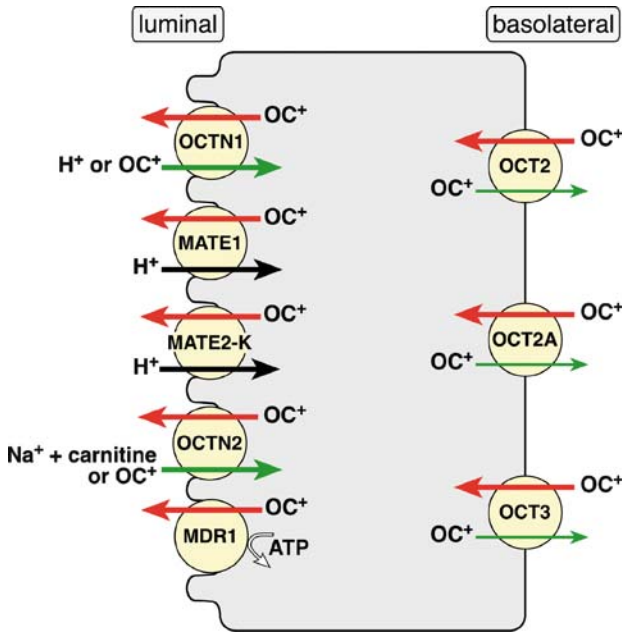

Fig. 4. Organic cation transporters in plasma membranes of human renal proximal tubules. For explanation see Fig. 2. *Red arrows* indicate transport activities that are involved in cation secretion whereas *green arrows* indicate transport activities involved in cation reabsorption. MATE1 and MATE2-K are secondary active proton–cation antiporters. OCT2A is a splice variant of OCT2. The basolateral localization of OCT3 was observed in unpublished experiments of K.S. Lips, W. Kummer, G. Ciarimboli, E. Schlatter., and H. Koepsell.

Biomedical implications. Polymorphisms and mutations in hOCT1 that lead to decreased activity of hOCT1 in the liver will impair the biliary excretion of hydrophobic cationic drugs. Defect mutations of the transporters MDR1 and/or MATE1 in the biliary membrane will result in increased intracellular concentrations of cationic drugs that are substrates of hOCT1 and/or hOCT3. This may lead to hepatotoxicity and hepatic tumors.

Kidney

In the kidney organic cations may be ultrafiltrated in the glomeruli or secreted in renal proximal tubules. Hydrophilic

organic cations that do not bind to plasma proteins are readily ultrafiltrated and may be reabsorbed in the proximal tubule. The reabsorption is dependent on the concentrations of the respective cation in the blood and the primary filtrate. For example, in rabbit and dog choline is secreted at high plasma concentrations, whereas it is reabsorbed at normal plasma concentrations below 25 μM (210,211). Many endogenous cations and cationic drugs are bound to plasma proteins and are not filtrated efficiently, and many of these are secreted actively. Secretion and reabsorption of organic cations may occur in proximal tubules, distal tubules and collecting duct, however, secretion in the proximal tubule has been mainly investigated (212–216). In a first step during secretion of organic cations in the proximal tubule, cations are translocated across the basolateral membrane. In humans OCT2 and OCT3 are supposed to be important for basolateral uptake whereas in rats OCT1, OCT2 and OCT3 may be involved ((45,61,65,132) and unpublished data of Lips K.S., Kummer W., Ciarimboli G., Schlatter E., and Koepsell H.) (Fig. 4). In human kidneys an alternatively spliced variant of hOCT2 (hOCT2A) that lacks the three C-terminal TMDs and exhibits some transport activity for TEA, MPP and cimetidine, may contribute to basolateral uptake (25). Since it has been shown that the organic anion transporter hOAT3 (SLC22A8) translocates the weak base cimetidine some organic cations may be also taken up by this transporter (5,217,218). In the second step of secretion, organic cations are released across the luminal membrane by the proton/cation exchangers MATE1, MATE2-K and/or OCTN1 (7,31,42). The proton/cation antiport mechanism used by these transporters helps to overcome the membrane potential during cation efflux. This step is energized by an extracellular> intracellular proton concentration difference that is generated by the sodium/proton exchanger (10) and, to a lesser extent by a V-type proton ATPase in the luminal membrane (219).

Biomedical implications. Renal excretion appears to be one of the main determinants for the pharmacokinetics of hydrophilic cationic drugs. The physiological importance of hOCT2 and hOCT3 for renal excretion of organic cations is suggested by the observation that no mutations of hOCT2 and hOCT3 could be identified that lead to largely impaired transport function and/or expression of these transporters. hOCT2 and hOCT3 in the proximal tubule are sites for clinical important drug–drug interactions. For example, therapeutic doses of cimetidine retard the renal elimination of procainamide (220,221). hOCT2 may be important for the renal excretion of the neurotransmitters dopamine, epinephrine and serotonin whereas OCT3 is supposed to be important for renal excretion of epinephrine, histamine, and norepinephrine (Table II). hOCT2 in the kidney appears to be critically involved in the renal excretion of agonists and antagonists of various receptors, of various blockers of ion channels and transporters and of various other drugs including a variety of psychoactive compounds. For example, hOCT2 is important for the renal excretion of metformin, a biguanide that is used to treat type-II diabetes (222) and polycystic ovary syndrome (223,224) and for the renal secretion of the cytostatic drug cisplatin (114). Metformin is mainly eliminated by glomerular filtration and tubular secretion, but part of it is also excreted in the bile. Decreased renal excretion of metformin during renal failure may lead to increased plasma levels. Increased concentration of metformin in the liver may lead to extensive inhibition of mitochondrial respiratory enzymes and may cause lactic acidosis, a rare but life-threatening side effect of biguanides. Administration of metformin to patients in combination with drugs that inhibit hOCT2 in the kidney but not hOCT1 in the liver may increase the risk of lactic acidosis. Cisplatin is an effective anti-neoplastic drug, but is also highly nephrotoxic. A combination of cisplatin with drugs that inhibit hOCT2 may help to prevent nephrotoxic side effect during treatment with cisplatin.

Lung

Nearly all polyspecific organic cation transporters are expressed in the lung. In humans OCT1, OCT2, OCT3, OCTN1 and OCTN2 are expressed in the respiratory epithelium of trachea and bronchi (47,225), OCT3 is additionally expressed in smooth muscle cells of bronchi and blood vessels (62). OCTN1, OCTN2, OCT1 and OCT3 are also expressed in alveolar epithelial cells (225,226). In differentiated ciliated cells of human bronchial respiratory epithelium, OCT1, OCT2, OCT3, hOCTN1 and OCTN2 are located in the luminal membrane. At variance, OCT2 and OCT3 have been detected all over the plasma membrane in the basal cells and intermediate cells (47). In rodents OCT1, OCT2 and OCT3 were detected in the luminal membrane of ciliated cells.

Biomedical implications. The polyspecific cation transporters OCT1 and OCT2 in the pulmonary respiratory epithelium are probably involved in non-neuronal autocrine and paracrine cholinergic regulation that influences beat frequency of cilia and regeneration of the epithelial cells. Bronchial ciliated epithelial cells have a relatively high cytosolic concentration of acetylcholine but do neither contain storage vesicles for acetylcholine nor the vesicular acetylcholine transporter that loads such vesicles (47,62). Since hOCT1 and hOCT2 are able to translocate acetylcholine in both directions across the plasma membrane they are supposed to mediate the release of acetylcholine across the luminal membrane. This function may be blocked by inhalational drugs. For example, in oocytes expressing hOCT2, the efflux of acetylcholine via hOCT2 was inhibited by micromolar concentrations of the inhalational glucocorticoide budesonide (47). OCT2 and OCT3 in the basal cells of the respiratory epithelium and/or OCT3 in smooth muscle cells of bronchi and blood vessels may influence the interstitial concentrations of neurotransmitters such as epinephrine, histamine and/or serotonin (Table II). Although the specific physiological and biomedical roles of individual polyspecific organic cation transporters in the lung are not understood we believe that the polyspecific organic cation transporters play a significant role for the distribution of cationic drugs in the lung. It is challenge to investigate whether organic cation transporters can be targets for the treatment of pulmonary diseases.

Brain

In brain of rodents and/or human expression of OCT1, OCT2, OCT3, OCTN1, OCTN2 and MATE1 has been reported (Table I). OCT2 is expressed in neurons (63), OCT3 is expressed in neurons and glial cells (67–69), and

OCTN2 has been located to glial cells (82). So far, detailed data have been only reported concerning the distribution of OCT3 in rat brain (44,72–74). In brain of rodents OCT3 has been assigned to the hippocampus (44,72), to the dorsomedial hypothalamus (73), to the area postrema and subfornical organ (74), and to ependymal cells of the third ventricle (73,74). In rat or mice data have been presented suggesting that OCT3 participates in serotonin uptake in brain and is involved in the modulation of motoric activity, of behavior, of regulation of salt intake, and of methamphetamine sensitization. (1) In one study in which OCT3 was downregulated in mice by infusion of antisense oligonucleotides into the third ventricle, the immobility time in the forced swimming test was reduced and methamphetamine-induced locomotor activity was increased (227). (2) Application of OCT-inhibitors to the medial hypothalamus in rats via microdialysis resulted in a reversible dose-dependent increase of extracellular serotonin concentration which was associated with increases in the grooming behavior (228). Since the affinity of serotonin to rOCT1 is 25times higher compared to rOCT3 (Table V), and rOCT1 is also expressed in rat brain this effect could be due to inhibition of serotonin uptake by rOCT1 rather than rOCT3 or rOCT2. (3) OCT3 deficient mice showed an increased ingestion of hypertonic saline under thirst (74). This suggests that OCT3 or proteins with changed expression after OCT3 removal are involved in the regulation of salt uptake. (4) When rats were behavioral sensitized to methamphetamine the expression of OCT3 was downregulated in brain, kidney and lung, and the renal excretion of amphetamine was decreased (151). Thus, it may be speculated that changes in the expression of OCT3 in brain and kidney may be involved in behavioral sensitization to methamphetamine. This speculation is supported by a recent study in which an assoziation between gene polymorphisms in *SLC22A3* and methamphetamine use disorder was demonstrated for persons taking multiple drugs (182). (5) In a recent study uptake of organic cations into cells isolated from the dorsomedial hypothalamus could be partially inhibited by typical OCT inhibitors including corticosterone (73). The authors speculated that corticosterone-induced inhibition of OCT3 may mediate stress-induced accumulation of serotonin in the dorsomedial hypothalamus and thereby modulate physiological and behavioral responses. This speculation is inconsistent with the observation that the IC_{50} value determined for inhibition of histamine uptake into hypothalamic cells by corticosterone was more than 100 times lower compared to the IC_{50} value determined for inhibition of cation uptake by corticosterone in oocytes or epithelial cells in which rOCT3 was overexpressed (see Table V, (72)).

Biomedical implications. The expression of OCTs in brain and the demonstration of the involvement of OCT3 in locomotor activity, salt intake and behavior strongly suggests the modulatory role of OCTs in brain functions. This role of OCTs is probably due to their effects on extracellular neurotransmitter concentrations and may be due to a modulation of membrane potential in neurons by the activity of these electrogenic transporters. Since many psychoactive drugs are substrates or inhibitors of OCT1-3 and are not only excreted by OCT1-3 but may also influence neuronal activity in brain by changing membrane potentials and/or neurotransmitter concentrations, the OCTs are targets for the treatment of central-nervous diseases.

Immune System

OCT1 and OCT3 have been identified in basophil granulocytes of mice (58) whereas human OCTN1 and OCTN2 were found in T lymphocytes and macrophages (36,75). Basophils play a crucial role during allergic diseases and helminth infections. They produce interleukin 4 (IL-4) and histamine which facilitate Th2 maturation (229). In immature basophils the newly synthesized histamine is not stored in granules but released to stimulate Th2 cell maturation. In bone marrow of mice a population of immature basophils with few granules was detected that contained mRNA of OCT1 and OCT3 (58). These basophils produce histamine together with IL-4 and IL-6 in response to growth factor like IL-3 and take up histamine from the environment (230). Uptake of histamine was inhibited by typical OCT inhibitors (58). Since the release of IL-4 and IL-6 from these cells was inhibited by extracellular histamine it was suggested that the production of IL-4 and IL-6 is regulated by the intracellular histamine. In OCT3 knockout mice uptake of radioactive histamine added to the exctracellular compartment was decreased whereas the intracellular concentration of newly synthesized histamine was increased. This is supposed to be due to blockage of OCT3 mediated influx of histamine or of OCT3 mediated efflux of histamine. Due to increased intracellular histamine in OCT3(−/−) cells the IL-6 production and release was decreased. The data suggest that OCT3 participates in the control of histamine and pro-Th2 cytokine synthesis that modulate immune responses. Consistent with this hypothesis is the observation that the concentration of histamine in the spleen of OCT3 (−/−) mice was increased compared to wildtype mice and that the survival rate of OCT3(−/−) mice in an experimental endotoxaemia model was largely decreased (231).

Biomedical implications. Treatment of patients with drugs with high affinity to OCT3, OCTN1 or OCTN2 may impair their immune response.

Tumors

hOCT1, hOCT2, hOCT3, hOCT6 and hOCTN2 have been detected in various tumors and/or tumor cells (54,87). These transporters translocate various cationic cytostatic drugs such as cisplatin that is transported by hOCT2 and rOCT2 (55,114,232,233), oxaliplatin that is transported by hOCT2, hOCT3 and hOCT1 (55,233), and Bamet-UD2 (cis-diammine-biursodeoxycholateplatinum(II) that is transported by hOCT1 (234). They may be critical to reach therapeutical cytostatic concentrations during treatment of tumors. Each cytostatic drug may be taken up by several uptake systems expressed in a specific tumor cell and may be released by efflux pumps. For example colon carcinoma cells express several transporters that accept Bamet UD2 as substrate (54,234). These are the apical sodium-dependent bile salt transporter ABST (*SLC10A2*), organic anion transporting polypeptides OATP1A2 previously called OATPA (*SLCO1A2*), OATPC (*SLCO1B1*), OATP8 (*SLCO1B3*), the organic cation transporter hOCT1 (*SLC22A1*), the organic

solute transporter subunits OSTα (*MGC39807*), OSTβ (*MGC118958*), the multidrug resistance protein MDR1 (*ABCB1*), and the multidrug resistance related proteins MRP2 (*ABCC2*) and MRP3 (*ABCC3*). In colon carcinoma ASBT, OSTα, OSTβ, MDR1 and MRP2 are expressed at low levels whereas OATP1A2, OATP-C, OATP8, hOCT1 and MRP3 are expressed at relatively high levels (54). During neoplastic transformation of the colonic epithelial cells the expression of drug transporters may remain about constant (ASBT, OSTα, hOCT1, OATP8, MDR1, MRP2, MRP3), may decrease (OSTA, OSTβ) or may increase (OATPC).

Biomedical implications. Knowledge concerning the expression of drug transporters in tumor cells may help to develop cytostatic drugs that are targeted to specific tumors. Good candidates for targeting transporters are those that are highly expressed and/or upregulated in tumors. hOCT1 may be useful to target oxaliplatin or Bamet 2 to colonic carcinomas. Since very high expression of hOCT2 has been detected in some tumor cell lines (87) hOCT2 may be useful to target cationic cytostatic drugs to specific tumors. Noteworthy, hOCT6 that translocates the cytostatic doxorubicin is expressed in various tumors and tumor cell lines (87). Toxicity of cytostatics for specific organs or functional systems may be prevented if different combinations of transporters are responsible for drug uptake into the tumor cells and for a specific toxic effect. In this case the toxic effect on normal cells may be prevented by comedication with a drug that inhibits uptake of the cytostatic drug into normal cells but does not influence uptake into the respective tumor cells.

Outlook

The identification of polyspecific transporters that are involved in absorption and excretion of organic cations has been almost completed. For most transporters various endogenous compounds and drugs have been tested for transport, stably transfected cell lines have been generated, and specific antibodies have been raised. For some polyspecific organic cation transporters the functional mechanism, the tissue distribution, and the *in vivo* function have been studied in detail, and for several transporters knockout mice have been generated. It has been recognized that polyspecific cation transporters are not only responsible for the intestinal absorption and hepatic and renal excretion of drugs, but also play important roles for the function of various physiological systems. Examples are neurotransmitter reuptake in brain, acetylcholine release during extraneuronal cholinergic regulations, and regulation of histamine release from basophils. Recent experiments indicated the potential role of organic cation transporters in various diseases.

For development of new cationic drugs we recommend to identify the polyspecific cation transporters that translocate the specific compound in humans and in the animal species that will be employed for drug tests *in vivo*. The identification of transporters can be easily performed using the available panel of overexpressing cell lines. After transporters have been identified that translocate a specific drug, good hypotheses can be made concerning the routes of excretion, the tissue distribution, concentrations in specific cells, and the interaction with other drugs. Importantly the in vitro studies with cell lines will help to identify potential species differences in pharmacokinetics and the appropriate animal model can be chosen for in vivo studies. In addition, it can be evaluated whether polymorphisms of certain transporters may influence the pharmacokinetics of the new drug and may lead to adverse drug reactions. In such cases genetic tests may help to adjust dosage or to exclude patients with critical mutations from the treatment with the respective drug.

Recently, new topics with high potential biomedical importance have been highlighted in cation transporter research. These are (1) research on the regulation of polyspecific organic cation transporters that may help to alter transporter expression or activity for therapeutical reasons, (2) the potential role of polyspecific organic cation transporters for behaviour and mental diseases, (3) the potential role of polyspecific organic cation transporters for the treatment of tumors, (4) the potential role of organic cation transporters for treatment of lung diseases, and (5) the potential role of organic cation transporters for immune reactions. Taken together, we have just started to unravel the physiological functions of the individual polyspecific organic cation transporters and to elucidate how their function can be employed for the benefit of humans during health and diseases.

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