Giuliano Ciarimboli · Sophie Gautron
Eberhard Schlatter *Editors*

Organic Cation Transporters

Integration of Physiology, Pathology, and Pharmacology

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This book is dedicated to the memory of Karl Julius Ullrich (1925–2010), former head of the Department of Physiology at the Max Planck Institute of Biophysics in Frankfurt am Main, Germany. Professor Ullrich was one of the founders of the renal transport physiology, especially with regard to the transport of organic anions and cations. Ullrich's department stood out as a beacon of transport physiology. Many of his scholars were able to further increase knowledge of transport physiology. In this way, we would like to express our admiration and gratitude for his outstanding work.

Preface

Transport across the cell membrane is essential for vital processes like entry of nutrients into the intracellular compartment, delivery of cellular products to extracellular and intracellular destinations, and handling of metabolism waste products and toxic substances and is necessary to keep the intracellular milieu constant. Transport across cell membranes is mediated by a variety of different transport proteins. This book focusses on transporters for organic cations, which are not directly energy-dependent, such as organic cation transporters (OCTs), organic zwitterions/cation transporters (OCTNs), and multidrug extrusion proteins (MATEs). Because these transporters are polyspecific, they accept many different substrates of endogenous (e.g. choline, acetylcholine, histamine, and monoamine neurotransmitters) as well as of exogenous (e.g. drugs like metformin, quinine, cimetidine, and cisplatin) origin.

Since the cloning of the first transporter for organic cations (rOCT1) in 1994, profound understanding of their structure, transport properties, and regulation has been obtained. In organs expressing these transporters at high levels, such as the intestine, liver, and kidney, transporters for organic cations play a pivotal role not only in absorption and in excretion of xenobiotics but also in their accumulation and toxicity. However, their expression is not restricted to organs typically involved in the transport of xenobiotics, but is found also in other tissues, such as the brain and reproductive organs. Recent studies with genetically modified animals have helped to unveil novel physiological, pathophysiological, and pharmacological roles of transporters for organic cations. While there is no doubt about the pharmacological and toxicological implications of transporters for organic cations for the organism, their physiological functions had remained largely elusive. Moreover, gender- and species-specific differences in the expression and properties of these transporters as well as the role of single nucleotide polymorphisms on their function have become a focus of attention in physiology, pathophysiology, and medical care.

This book presents current knowledge on the expression, physiological functions (see Chap. 1 by G. Ciarimboli), and regulation (see Chap. 5 by E. Schlatter and Chap. 6 by L.M. Aleksunes) of transporters for organic cations in various organs, on

their gender and species dependencies (see Chap. 9 by I. Sabolić, D. Breljak, and T. Smital), and on their role in pathophysiological situations. This overview should be of high interest for researchers and students in various areas of integrative, organ, cell, and molecular physiology and will contribute to delineate an integrative physiological interpretation of transporter function.

Another important aspect of the book is that it conjugates integrative transporter physiology with structural and molecular biology (see Chap. 2 by H. Koepsell and T. Keller), genetics (see Chap. 4 by M.V. Tzvetkov, N. Dalila, and F. Faltraco), pharmacology, and pathophysiology (see Chap. 3 by K. Inui and H. Motohashi and Chap. 8 by K. Tieu), offering an integration of the knowledge in these fields. The different chapters of the book present the state of the art of the research in these different fields. For this reason, the book addresses both expert readers and readers with a more general interest in understanding transporter function in physiology and pathophysiology. Hence, the book should also attract people interested in adaptive mechanisms of the organism to conditions, such as salt intake, anxiety, and stress (see Chap. 7 by A. Orrico and S. Gautron).

Since up to 40 % of the prescribed drugs are organic cations, this book will provide important information on the involvement of transporters for organic cations in determining specific effects but also side effects induced by particular drugs, offering new approaches for a successful translation from physiology to clinical therapy. Finally, because of the expression of transporters for organic cations in plants, the role of these transporters for the environmental cycling of pharmaceutical residues is also presented (see Chap. 10 by T. Eggen and C. Lillo).

In conclusion, we think that a book concentrating on the latest developments of integrative, organ, cell, and molecular aspects of function of transporters for organic cations will furnish an optimal platform to integrate the knowledge on these transporters and obtain a more comprehensive physiological understanding of their function.

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GC would like to thank Frank Thevenod for the support in organizing the symposium on organic cation transporters, whose idea came up when visiting together with him.

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Eberhard Schlatter is Full Professor at the faculty of medicine of the University of Münster, Germany. He studied Biology at the University of Hannover, Germany. In 1981, he received a Ph.D. in Biology at the University of Hannover, Germany. In 1989, he habilitated in Physiology at the University of Freiburg, Germany. In 1993, Eberhard Schlatter was appointed Full Professor for Experimental Nephrology at the Department of Nephrology and Hypertension of the University Clinic Münster, Germany.

His main research interests since then are focused on renal effects of cGMP activating peptide hormones, rejection processes in experimental kidney transplantation, and, most recently, characteristics of organic cation transporters, their interaction with drugs, and their role in drug toxicities.

Dr. Sophie Gautron is a permanent researcher at the Inserm (Institut National de la Santé et de la Recherche Médicale). She holds a PhD degree in Molecular and Cellular Genetics from Pierre et Marie Curie University and an HDR degree from Paris-Est Créteil University. She is appointed at the Neuroscience Paris Seine laboratory (CNRS UMR8246/Inserm U1130/UPMC UMCR18) in Paris where she co-leads of the "Physiopathology of Psychiatric Diseases" Team. Her recent work focuses on the function of low-affinity monoamine transporters in the central nervous system, with a particular interest in psychiatric disorders such as depression and addiction in preclinical models.

Chapter 1
Introduction to the Cellular Transport of Organic Cations

Giuliano Ciarimboli

Abstract Organic cations (OCs) are substances of endogenous and exogenous origin to which belong important neurotransmitters such as histamine and serotonin and also drugs such as metformin. Because OCs are positively charged they need membrane transporters to permeate the plasma membrane. Membrane transporters which translocate OCs according to their electrochemical gradient belong to the Solute Carrier (SLC) families 22 (organic cation transporters (OCT) 1–3, and organic cation transporters novel (OCTN) 1–2) and 47 (multidrug and toxin extrusion (MATE) 1–2). This chapter collects the information on expression and function of these transporters present in the literature, comparing the characteristics of human and rodent transporters. These data show that OCTs play an important physiological role for neurotransmitter balance in the body. Moreover, they are also important uptake routes for intracellular drug delivery and, considering their high expression in excretory organs, together with MATEs are responsible for drug excretion. For this reason, OCTs and MATEs can be important determinants of drug efficacies and also toxicities. OCTNs are transporters involved in the cellular uptake of substances, which are important in cell metabolism and in signal transmission, such as ergothioneine, carnitine and acetylcholine. Even though the expression and function of orthologs of transporters for OCs is generally similar, still there are important differences that have to be considered for a proper interpretation of translational studies. Paralogs of transporters for organic cations often display similar characteristics, however they show also important differences e.g. with regard to interaction with substrates and to regulation. Other important functional aspects of transporters for organic cations, such as the molecular correlates of polyspecificity, regulation, interaction with drugs, genetic variations, role in the central nervous system, and distribution in the plants are discussed in the other sections of this book.

Keywords Organic cations • Transporters • Neurotransmitters • Drugs • Plasma membrane

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Introduction

The development of a plasma membrane was a fundamental step in the evolution of the cell, because it allowed the separation of an internal milieau from the external environment, which is of special importance to protect the genetic material. However, this important evolutionary progress created new challenges, because now the cell had to find solutions able to guarantee the entry of all essential nutrients into the cytoplasmatic compartment, the distribution of cellular products such as proteins, complex carbohydrates and lipids into and beyond the plasma membrane, and the handling of waste products and toxic substances, processes aimed at keeping the intracellular milieau constant [1]. The solution of these problems was the development of specialized transport systems of proteinic nature (transporters) embedded in the plasma membrane. Thus, it is evident that transporters are essential to sustain life and adaptation to changes in the environment. Their malfunction can result in diseases and, therefore, they are target of therapeutic intervention. Some transporters are also responsible for efficacy and also dangerous side-effects of chemotherapy [2, 3].

A total of 40,678 transport proteins classified into 134 families were predicted by whole-genome transporter analysis of 141 species, including 115 Eubacteria, 17 Archaea and 9 Eukaryota [4]. Eukaryotic cells, especially those of multicellular eukaryotic organisms, express the largest total number of transporters, which display a high number of paralogs generated by gene duplication or expansion within certain transporter families. The formation of paralogs is a sign of specialization, since closely related paralog transporters become expressed in specific tissues or at specific subcellular localisation and developmental time points [4].

Based on mode of transport and energy-coupling source, molecular phylogeny, and substrate specificity, there are five main recognised classes of transporters: pores and channels, electrochemical-potential-driven transporters, primary active transporters, group translocators, and transmembrane electron carriers ([1], http://www.tcdb.org). Each transporter category is further classified into individual families and subfamilies (Table 1.1).

This book focuses on transporters for organic cations, which are not directly ATP dependent and mediate the substrate movement through the plasma membrane according to the electrochemical gradient. According to the "*Transporter Classification Database*" (http://www.tcdb.org), these transporters belong to the family 2, subfamily 2.*A* (Table 1.1). Here a special attention will be payed at organic cation transporters (OCTs), novel organic cation transporters (OCTNs), and multidrug and toxin extrusion transporters (MATEs).

Basing on the amino acid sequences, the Human Genome Organisation (HUGO), classified human transporters in 54 **Sol**ute **C**arrier (SLC) families (a transporter has been assigned to a specific family if it has at least 20–25 % amino acid sequence identity to other members of that family [5]). These SLC families comprise 386 different SLC human transporters [6], additional new members being identified constantly [5].

Table 1.1 Transporter classification (classes and subclasses) according to the International Union of Biochemistry and Molecular Biology (http://www.tcdb.org)

1. Pores and channels	1.A α-Helical channels
	1.B β-Strand porins
	1.C Pore-forming toxins
	1.D Non-ribosomally synthesized channels
	1.E Holins
These proteins catalyze facilitated diffusion by passage through a transmembrane aqueous pore or channel. They do not exhibit stereospecificity but may be specific for a particular molecular species or class of molecules	
2. Electrochemical-potential-driven transporters	2.A Transporters or carriers (uniporters, symporters and antiporters)
	2.B Non-ribosomally synthesized transporters
These transporters utilize a carrier-mediated process not directly linked to a form of energy other than chemiosmotic energy to catalyze uniport (a single species is transported by facilitated diffusion), antiport (two or more species are transported in opposite directions) and/or symport (two or more species are transported together in the same direction)	
3. Primary active transporters	3.A P–P-bond-hydrolysis-driven transporters
	3.B Decarboxylation-driven transporters
	3.C Methyltransfer-driven transporters
	3.D Oxidoreduction-driven transporters
	3.E Light-driven transporters
These transporters use a primary source of energy (chemical, electrical and solar) to drive active transport of a solute against a concentration gradient	
4. Group translocators	4.A Phosphotransferases
Transport systems of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. The product of the reaction, derived from extracellular sugar, is a cytoplasmic sugar-phosphate. The enzymatic constituents, catalyzing sugar phosphorylation, are superimposed on the transport process in a tightly coupled process	
5. Transmembrane electron carriers	5.A Two-Electron Carriers
	5.B One-Electron Carriers
Systems that catalyze electron flow across a biological membrane, from donors localized to one side of the membrane to acceptors localized on the other side. These systems contribute to or subtract from the membrane potential, depending on the direction of electron flow. They are therefore important to cellular energetics	

According to this classification, OCTs and OCTNs belong to the SLC22 and MATEs to the SLC47 family (Table 1.2). The HUGO nomenclature system is also informally used with lowercase letters for rodents and this notation has been also extended to the spelling of protein (e.g., *Slc22a1* and Oct1 denote the rodent orthologs of the human *SLC22A1* gene and hOCT1 protein, respectively).

Many of the SLC families present in *H. sapiens* (among these also the SLC22 family) are highly evolutionary conserved in Bilaterian species [7]; moreover, the high representation of the SLC22 family in the plant *Arabidopsis thaliana*, suggests that it has an ancient origin [7]. More information about transporters for organic cations in plants will be presented in the Chap. 10 by T. Eggen and C. Lillo in this book.

Table 1.2 The SLC22A and SLC47A families

Gene name	Gene locus	Protein name	Function
SLC22A1	**6q25.3**	**hOCT1**	Electrogenic cation transport
SLC22A2	**6q25.3**	**hOCT2**	
SLC22A3	**6q25.3**	**hOCT3**	
SLC22A4	**5q23.3**	**hOCTN1**	Carnitine and cation transport
SLC22A5	**5q23.3**	**hOCTN2/CT1**	
SLC22A16	**6q21**	**hCT2/hFLIPT2/hOCT6**	
SLC22A6	11q12.3	hOAT1	Anion transport
SLC22A7	6q21.1	hOAT2	
SLC22A8	11q12.3	hOAT3	
SLC22A9	11q12.3	hOAT7	
SLC22A11	11q13.1	hOAT4	
SLC22A12	11q13.1	hURAT1	
SLC22A13	3p22.2	hOAT10	
SLC22A20	11q13.1	hOAT6	
SLC22A10	11q12.3	hOAT5	Predominant substrates not yet determined
SLC22A14	3p22.2	OCTL2/hORCTL4	
SLC22A15	1p13.1	FLIPT1	
SLC22A17	14q11.2	BOIT/BOCT	
SLC22A18	11p15.5	TSSC5/hORCTL2	
SLC22A23	6p25.2		
SLC22A24	11q12.3		
SLC22A25	11q12.3	UST6	
SLC22A31	16q24.3		
SLC47A1	**17p11.2**	**hMATE1**	H^+-coupled electroneutral exchange of organic cations
SLC47A2	**17p11.2**	***hMATE2***	

The transporters presented in this book are in bold characters

Substrates of Transporters for Organic Cations

The substrates of the three types of transporters for organic cations discussed in this book (OCTs, OCTNs, MATEs) are mainly organic cations, even though also inorganic substances such as Cd^{2+} [8] and cisplatin [9, 10] have been demonstrated to be accepted as substrate by some of these transporters. Moreover, some of these proteins can transport also zwitterions such as L-carnitine [11, 12] (OCTNs) and cephalexin and cephradine [13] (human MATE1, hMATE1) and anionic substances such as estrone sulphate (hMATE1, [13]), acyclovir, and ganciclovir (hOCT1 and hMATE, [13, 14]).

Organic cations (OCs) can derive from endogenous and also exogenous sources. Endogenous OCs are important neurotransmitters such as histamine, serotonin and dopamine [15] and polyamines such as putrescine and spermidine [16], which have an important function in many cellular processes such as DNA stabilization,

regulation of ion channel activity, gene expression, and cell proliferation [17]. In general, neurotransmitters and polyamines seem to be low affinity substrates of transporters for OCs, underlying the importance of these transport systems in places, where the concentration of such substances is high. Exogenous OCs are drugs (up to 40 % of the prescribed drugs are OCs [18]), xenobiotics such as the herbicide paraquat and the DNA intercalating agent ethidium bromide [19, 20], and also several natural contents of fungi, fruits and vegetables. Of practical experimental interest are fluorescent OCs such as 4(4-dimethylaminostyryl)-*N*-methylpyridinium (ASP^+), and rhodamine 123, which are substrates for several transporters for OCs and are therefore useful for investigating transporter activity [21–24].

OCs are also classified as type I and type II OCs depending on their chemical structure. Type I OCs are small (below 500 Da), strongly hydrophilic cations, such as tetraethylammonium (TEA^+) and 1-methyl-4-phenylpyridinium (MPP^+), while Type II OCs are large, more hydrophobic and mostly polyvalent substances, such as D-tubocurarine and quinine [25].

Even though many substrates are common between OCTs, OCTNs, and MATEs, every single transporter has a specific interaction spectrum with the substrates and inhibitors. For example, TEA^+ is a substrate for OCT1 and OCT2 [26], but not for OCT3 [15]. Some substances are known to bind to, but not to be transported by these transporters, as for example shown for proton pump inhibitors [27].

From this brief description it is evident why these transporters are called polyspecific. The translational relevance of studies on OCs with laboratory animals should be cautionally inferred, since rodent and human transporter orthologs can differ in substrate specificity, tissue expression [28] and also regulation (see Chap. 5 by E. Schlatter of this book), even though the global substrate preference of the SLC22 family seems to be conserved over a long evolutionary time [7].

Integration of OC Transport

Since many transporters for OCs are expressed in liver and kidney, they play a pivotal role in drug and xenobiotic absorption and excretion [29]. In these organs, SLC22A and SLC47 transporters are expressed in hepatocytes and renal proximal tubules cells, which are highly polarized cells, and mediate the coordinated movement of OCs across the cell by a concerted activity, mainly resulting in excretion of OCs into bile or urine. The first step for hepatic and renal OC secretion is their absorption from the basolateral side into the cells. While in human kidney this process is mainly mediated by OCT2 (Fig. 1.1), in rodent kidney it is supported by Oct1 and Oct2. OCT3 shows only a tiny expression in the basolateral membrane of proximal tubule cells, and for this reason is probably less important than OCT2 under normal conditions. OCs are secreted in a second step from the tubular cell into the tubular lumen. In the kidney this process is mediated by different transporters: the Na^+-carnitine cotransporter OCTN2, and *P*-glycoprotein (also named MDR1), an ATP-dependent transporter that probably mediates the efflux of bulky

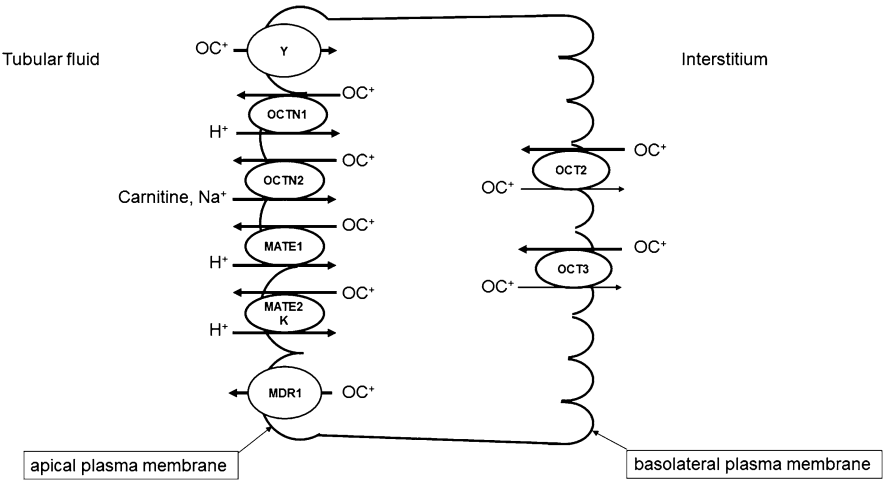

Fig. 1.1 Transport systems for organic cations in human renal proximal tubules. The basolateral uptake of organic cations (OCs) from interstitium is mainly mediated by hOCT2, where there is also a much lower expression of hOCT3. Secretion of OCs into the tubular fluid is mediated by MATE1, MATE2K, and OCTN1 in exchange with H^+. The necessary H^+ gradient is substained by the activity of NH3, an apically expressed Na^+/H^+ exchanger (not shown). Bulky OCs are secreted into the urine under energy consumption by the Multidrug Resistance protein 1 (MDR1). OCs can be also reabsorbed from the tubular fluid by an not yet identified transport system (Y), and then transported into the interstitium by OCT. Modified from Koepsell et al. [30] and Ciarimboli and Schlatter [31]

hydrophobic OCs, and other H^+/organic cation antiporters (OCTN1, MATE1, and MATE2K in Fig. 1.1). According to their electrochemical gradient, in the kidney OCs can be also reabsorbed from the lumen into the interstitium. For this process, a polyspecific cation transport system mediating their uptake across the luminal membrane of proximal tubular cells has been proposed, but not yet molecularly identified (system Y in Fig. 1.1). The efflux across the basolateral membrane into the interstitium may be mediated by OCTs. The hepatic transport pathways of OCs in humans are illustrated in Fig. 1.2. The uptake of OCs into human hepatocytes is mediated by OCT1 present on the sinusoidal membrane. The extrusion of OCs in the canalicular space is mediated by *P*-glycoprotein (MDR1 in Fig. 1.2) and MATE1.

Genetic Organisation of Transporters for Organic Cations

Some of the *SLC22A* genes (e.g. the genes for OCT1 and 2, OCTN1 and 2, and also OAT1 and 3) are organized in the mouse and in humans as tightly linked pairs [32]. The gene coding for OCT3 is also in close proximity of the *SLC22A1-2* pair, and also *SLC47A1* and *SLC47A2* are adjacent. The gene pairing probably originates

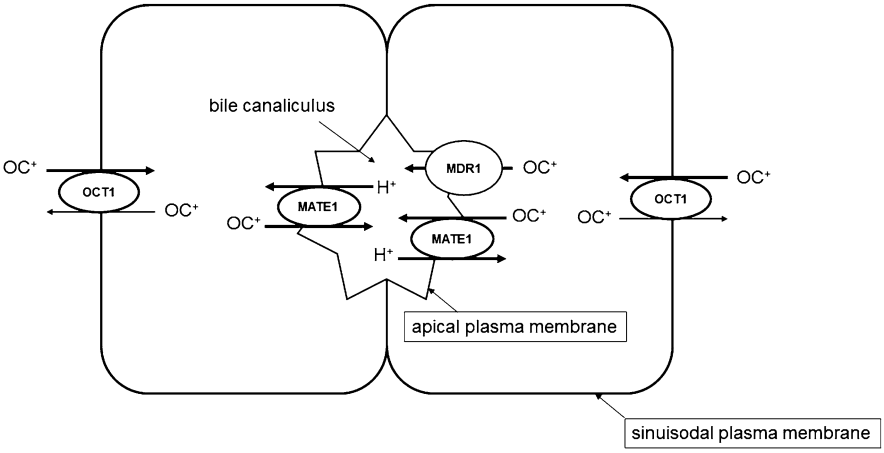

Fig. 1.2 Transport systems for organic cations in human hepatocytes. OCs are transported through the sinusoidal membrane (corresponding to the basolateral side) of hepatocytes by hOCT1. Secretion of OCs into the bile canaliculus is mediated by MATE1 expressed in the apical membrane in exchange with H^+. Bulky OCs are secreted into the bile under energy consumption by MDR1. Modified from Koepsell et al. [30] and Ciarimboli and Schlatter [31]

from an evolutionary duplication event, aimed at conferring the advantages of redundancy or broader substrate specificity [33].

The genes encoding for Oct1-3 are clustered within a 300-kb genomic region between the insulin-like growth factor receptor 2 (*Igf2r*) and the *Plg* (plasminogen) genes on mouse chromosome 17 and on rat chromosome 1. Also in humans, the genes encoding for OCT1-3 are clustered in a region between the *IGF2R* and the *APO*(*a*)*-like* genes on chromosome 6 [34].

Interestingly, expression of *Slc22a2* and *Slc22a3* in mouse placenta is predominantly maternally imprinted, at least till embryonic day 15.5 for *Slc22a3* [35]. Imprinting is an epigenetic modification, which leads to preferential expression of a determined parental allele in somatic cells of the progeny. After evolutionary divergence, imprinting of only 29 transcripts has been conserved in mice and humans [36]. Imprinted genes often have key roles in embryonic development, but also in postnatal functions including energy homeostasis and behaviour [37]. In humans, imprinting of the *SLC22A2* and *SLC22A3* genes in the placenta is not a general phenomenon, but is present only in few subjects with a temporal expression pattern resembling that of the murine genes [38].

Topology of Transporters for Organic Cations

The transporters of the SLC22 family have a similar predicted membrane topology consisting of 12 alpha-helical transmembrane domains (TMDs), a large glycosylated extracellular loop between the first and the second TMD, and a large intracellular

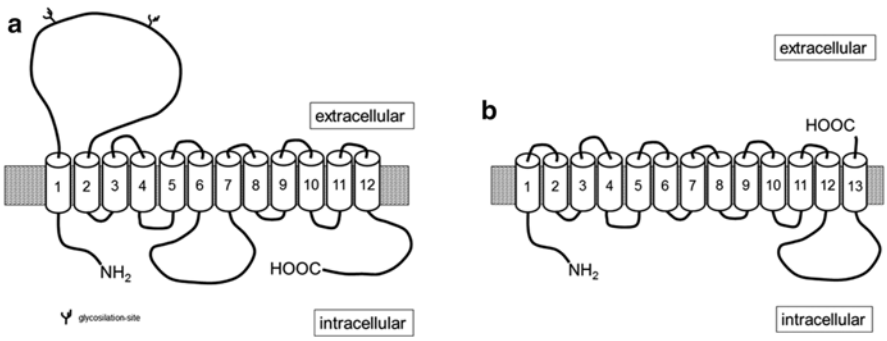

Fig. 1.3 Panel (**a**) shows the proposed secondary structure of OCTs and OCTNs. These transporters have 12 TMD, a big extracellular and a big intracellular loop with type and subtype specific glycosylation and phosphorylation sites, respectively. Amino- and carboxy-termini are intracellular. Panel (**b**) shows the proposed secondary structure of MATEs. These transporters have 13 TMD, an intracellular and an extracellular terminus. Modified from Ciarimboli and Schlatter [31]

loop between TMDs 6 and 7 with consensus sequences for phosphorylation (Fig. 1.3a). Both the amino- and carboxy-termini are intracellularly localized.

The topology of SLC47 transporters seems to be somewhat different, as these transporters possess 13 TMDs, an intracellular amino- and an extracellular carboxy-terminus, no glycosylation sites and few intracellularly located putative phosphorylation sites (Fig. 1.3b) [39, 40]. However, there are data demonstrating that the functional core of MATE1 consists of 12 TMDs [41].

In the following the basic information on OCT, OCTN, and MATE present in the literature will be summarized, focussing on human and rodent transporters, which will be separately described, because of the known differences between species.

Organic Cation Transporters (OCTs)

Transport of organic cations by the three OCT subtypes (OCT1, OCT2, and OCT3) is electrogenic, Na^+- and H^+-independent and bidirectional [29]. The driving force is supplied exclusively by the electrochemical gradient of the substrate. The first member of the SLC22 transporter family was isolated and identified by expression cloning from rat kidney and was named rat organic cation transporter 1 (rOct1) [42]. In this initial study, it was shown that rOct1 has functional characteristics resembling those of the organic cation transport processes previously described in the basolateral membrane of renal proximal tubule cells and of hepatocytes. Mammalian orthologs of *OCT1* have been cloned also from human [43, 44], rabbit [45], and mouse [46].

Mouse Organic Cation Transporter 1 (mOct1)

The gene *Slc22a1* encodes for a 556 amino acids (aa) protein, which is mainly expressed in the liver and the kidneys [46, 47]. Upstream sequences for *mOct1* contain putative binding motifs for hepatocyte (HNF5 and H-APF-1), and mammary (WAP and MGF) specific expression, and potential binding sites for metallothioneine-regulated gene expression (MBF-1, GR-MT-IIA, and AP-2) [48]. *Slc22a1* transcripts have been shown to turn up in the mouse kidney at midgestation, at the time when the proximal tubules begin to differentiate, and to increase gradually in the course of nephron maturation. *Slc22a1* transcripts are also transiently expressed in other tissues than the kidneys such as the ascending aorta and the atrium [49]. In the liver, ontogenic expression data showed that *Oct1-3* approach adult expression levels at an age of about 3 weeks [50]. The highest hepatic Oct1 mRNA labelling intensity was detected in the hepatocytes which are localized in the proximity of the vena centralis, while in the kidney Oct1 mRNA appeared to be unevenly distributed throughout the renal cortex but not in glomeruli [51]. The mOct1 has been found to be higher expressed than mOct2 and mOct3 in the S1, S2, and S3 segments of the proximal tubules (relative mRNA expression of Oct1/Oct2/Oct3: 1/0.3/0.01) [52]. Expression and function of mOct1 has been detected also in other organs: in the luminal blood-retina barrier [53] Oct1 and Oct2 have been found to be expressed in an age-dependent manner (with decreased expression in aged mice [54]) in endothelial cells of mouse brain microvessels (BMVs). Elevated Oct1 mRNA levels were measured in mammary glands of lactating mice, suggesting that this transporter may be involved in the transfer of drugs into milk [55].

Generally, when expressed in polarized cells, such as hepatocytes and proximal tubule cells, mOct1 localizes to the basolateral plasma membrane [56]. However, in enterocytes this transporter has been shown to be expressed on the apical plasma membrane [57].

The transport mediated by mOct1 has been demonstrated to be pH- and Na^+-independent and potential dependent [58]. In mice, Oct1 and Oct2 have been identified also in the respiratory epithelium, where they seem to be involved in the acetylcholine (ACh) release [59]. Interestingly, Oct1 and Oct3 have been also found to be expressed in mouse urothelium, where they may mediate ACh secretion [60]. Transport studies showed that the mOct1 mediates the uptake of choline with a K_m of 42 μM [61] and the low-affinity transport of serotonin [51]. Moreover, mOct1 accepts also exogenous OCs such as [^{14}C]-TEA^+ and MPP^+ as substrates (K_m = 38 μM [47] and 10 μM [62], respectively).

To better understand the physiological role of Oct1, Oct1 knockout (*Slc22a1*$^{-/-}$) mice were generated [63]. These mice were viable, healthy, and fertile and did not appear to have obvious phenotypic abnormalities; they only showed a decreased hepatic accumulation and intestinal excretion of exogenously administered TEA^+ [63]. Further studies with *Slc22a1*$^{-/-}$ mice showed that Oct1 is important for the hepatic and intestinal uptake of metformin, a hypoglycemic agent used for the oral treatment of type 2 diabetes mellitus, whereas its renal distribution and excretion are